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Review Article

# Establishment and Maintenance of Heritable Chromatin in *C. elegans*

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**Abstract**

Mounting evidence suggests that environmental exposures in one generation may result in changes in gene expression that are heritable for multiple generations, yet are unaccompanied by genetic mutations. These phenomena are considered to be the result of epigenetic mechanisms, which can establish metastable states of chromatin-based genome architecture that can be passed through the gametes into subsequent generations. Such heritability requires that normally transient epigenetic changes become stabilized in the germline and become part of the information that is passed from one generation to the next. Work in the genetic model organism *Caenorhabditis elegans* has uncovered a number of mechanisms that influence transgenerational epigenetic inheritance. These include non-coding RNA-based mechanisms that target genomic loci for heritable repression through the recruitment of histone modifiers. Less understood is how heritable gene activation may be established and maintained. In this review we summarize results that indicate that a network of antagonistic chromatin modifying activities may help maintain heritable gene transcription in the germline of C. *elegans*. Chief among these activities may be RNA Polymerase II, and the histone methyl transferases with which it is associated, which "mark" regions of transcriptional activity in germ cell chromatin. These patterns are then further maintained by transcription-independent mechanisms that are essential for germline function in subsequent generations.

## INTRODUCTION

### Epigenetic inheritance in the model system *C. elegans*

The term "epigenetics" is most strictly defined as heritable changes in gene expression not associated with changes in the DNA. Heritability is the key component of this definition, and assumes stability of the change through mitosis or meiosis. Generational inheritance, of course, relates to the latter; i.e., alterations of gene expression that initially occur in the parent, but are then stably observed in the offspring despite the genome encountering dramatic structural alterations during both meiosis and gametogenesis. Until recently, such stable epigenetic "memory" was considered to be largely the hallmark of differential DNA methylation, specifically cytosine methylation in the context of CG dinucleotides [1]. In contrast, other chromatin modifications, such as histone post-translational modifications (PTMs) were thought to be too unstable to provide heritable memory, given the dynamics of histone replacement during most genetic events, including DNA replication [2-4]. However, it has recently become clear that histone methylation, like DNA methylation, has both *de novo* establishment and stable maintenance mechanisms that contribute to epigenetic memory.

Histones are highly conserved proteins that form core particles, termed nucleosomes, around which the DNA is tightly coiled. Nucleosomes are histone octamers, each assembled from two histone H2A/H2Bdimers, together with a histone H3/H4 tetramer formed from two molecules each of histones H3 and H4. PTMs have been documented on all core histones, with most occurring at their N- and C-terminal tails. These modifications include: methylation of arginine (R) residues; methylation, acetylation, ubiquitination, ADP-ribosylation, and sumoylation of lysines (K); and phosphorylation of serines and threonines [5]. In this review, we will focus on methylation of specific lysines in histone H3: methylysines 4, 9, 27, and 36 (H3K4me, H3K9me, H3K27me, and H3K36 me, respectively). In general, H3K4me and H3K36me are associated with euchromatic or active chromatin states, while H3K9me and H3K27me are largely associated with heterochromatic or repressed chromatin states [6]. Although this would imply these two "classes" of histone marks should never co-exist, in mammalian embryonic stem cells (ESCs), certain developmental genes have been found to be dually marked by histone H3 lysine 4 tri-methylation (H3K4me3) and H3K27me3, and are termed "bivalent" [7,8]. Most ES cell gene promoters are enriched with unmethylated cytosines at CpG islands, and these tend to be marked by H3K4mewhether or not they are transcribed [9,10].A subset of these genesis also broadly marked by H3K27me; i.e., are bivalent [9]. It has been proposed that bivalency represents an in determinant state, in which these

repressed genes are poised for either transcription activation (loss of H3K27me3) or maintaining repression (loss of H3K4me3), depending upon which developmental path the cell ultimately engages [7,8]. However, an *in vitro* neuronal differentiation study found that although numerous bivalent domains follow this pattern of resolution to monovalency of H3K4me or H3K27me, numerous genes marked only by H3K4me in ESCs also gained H3K27me during differentiation to the neuronal progenitor state, indicating that bivalency can be dynamic [10]. Oddly, although most bivalent loci encode early somatic transcription factors, their bivalent state is also observed in spermatids, and their bivalent status is conserved in highly diverged species [11]. This is rather startling, as it suggests that these histone markings are not only preserved from gametogenesis to embryo, and possibly maintained from embryo back to gamete, but also that their epigenetic status is also under strong selection and is evolutionarily conserved. Inheritance of histone methylation is thus another component of the information that, in addition to the methylation state of the genetic material, is transmitted between generations.

Transgenerational epigenetics is thus the study of how epigenetic information is stabilized and passed through the germline from generation to generation. Perhaps because of its potential for inter-generational transmission, the epigenetic regulation of gene expression in the germline has many complexities that can differ from those in somatic tissues. Among these complexities is the maintenance of an underlying pluripotency despite engaging in gamete development. The roles of histone modifications during germline-differentiation, gamete specification, and transgenerational inheritance have been largely studied in model organisms [12]. Among these, the nematode *C. elegans* has provided an excellent model for transgenerational epigenetic studies because of its short life-span, deeply annotated sequenced genome, and a vast repertoire of available mutant strains and reagents. *C. elegans* researchers have provided insights into understanding the role of these epigenetic modifications in germline function, fertility and the transgenerational effects of altering the epigenome.

## *C. elegans* germline development

The germline is the only cell lineage that is passed to the next generation and hence it has been termed "immortal". In this way the germ cells link all generations and any alterations in genetic or epigenetic information happening in these cells can affect the progeny and subsequent generations. Hence proper maintenance of genetic and epigenetic information during germ-cell development and gametogenesis is necessarily under tight surveillance. The *C. elegans* germline is considered a 'preformistic' mode of specification; i.e. specialized maternal cytoplasm (germplasm) is asymmetrically partitioned to the posterior pole after fertilization, and similarly to the posterior germline precursor "P cells" (*P1, P2, P3,* and*P4*) at each of 4 subsequent asymmetric cell divisions. The last *P4* cell divides symmetrically and generates the two equivalent primordial germ cells named *Z2-Z3* (Figure 1A) [13]. The *Z2-Z3* cells undergo one round of DNA replication and arrest at G2/M phase for the rest of embryogenesis [14].

In many species, the establishment of the germline is accompanied by a period of transient suppression of mRNA production [15,16]. In *C. elegans,* RNA Polymerase II [17] activity is initially prevented in the P1-P4 germline precursors via inhibition by a maternal protein, PIE-1 [18]. PIE-1 appears to inhibit kinase activities that regulate Pol II initiation and elongation [19]. After the birth of Z2-Z3, however, PIE-1 is degraded and Pol II phosphoepitopes that correlate with transcription elongation are observed in Z2-Z3 [18,20]. Interestingly, this activity is transient, and the Pol II elongation-associated phosphoepitopes decline [21]. Oddly, the appearance of Pol II elongation-associated phosphoepitopes temporally overlaps with a dramatic reduction of another mark normally associated with transcription: H3K4me [22]. Only after hatching and larval feeding do *Z3-Z3* re-enter the cell cycle, begin to proliferate, and Pol II activation reappears [22]. H3K4me also reappears in the chromatin near the time of hatching [22] .

Initially, larval germ cell proliferation generates a pool of germline stem cells, which then support the continuous production of germ cells that will undergo meiosis and gametogenesis through the rest of larval development and in adults (Figure 1B) [23-26]. Germ cells in animals with XX sex chromosome karyotype undergo hermaphrodite development: the first germ cells completing meiosis in the L4 stage larvae develop as sperm, and those completing meiosis as adults produce oocytes. These animals are self-fertile, with oocytes produced in adults fertilized by the sperm produced as L4 larvae. Germ cells produced in XO animals only generate sperm; and these XO animals develop as morphologically distinct males capable of mating with and fertilizing hermaphrodite [26,27]. Importantly, the X chromosomes in XX germ cells go through meiosis with a homologous partner, whereas the X in male meiosis lacks a partner. This has epigenetic consequences for the male X, as well as what appear to be evolved consequences for the genetic content of the X, as will be detailed below.

## Epigenetic memory in the Germline I: H3K27 Methylation

The epigenetic landscape in the *C. elegans* germline is established and maintained by multiple distinct mechanisms, including those that perform *de novo* methylations of histone H3, and those that stabilize and maintain pre-existing patterns. The maintenance modes are illustrated by the *maternal effect sterile (mes)* genes, which as their names imply are required to be functional in the mother for fertility of the offspring. These genes encode maternally provided histone methyl transferase activities that maintain 'epigenetic germline memory' in the progeny. One set of MES proteins, MES-2, MES-3 and MES-6, together form a worm version of the conserved Polycomb repressor complex 2 (PRC2) that is required for H3K27 methylation [28]. Maternal supply of the MES proteins is both necessary and sufficient for fertile progeny; depletion of any of them leads to germ cells that die after a few post-embryonic divisions of Z2/Z3 [29]. Maternally inherited MES proteins are visibly enriched in the embryonic Z2/Z3 cells, yet they become quickly diluted through cell division in the post-embryonic germ cells [30,31]. Despite a presumed lack of continued MES function, the mutant germline stem cells produce hundreds of fully functional gametes and viable (but sterile) offspring (Figure 2) [29]. This suggests that PRC2, and the H3K27me3 it produces in germ cell chromatin, is only required in the embryonic germ cells for normal control of gene expression in the many hundreds of post-embryonic germ cells that are descended from the embryonic cells.

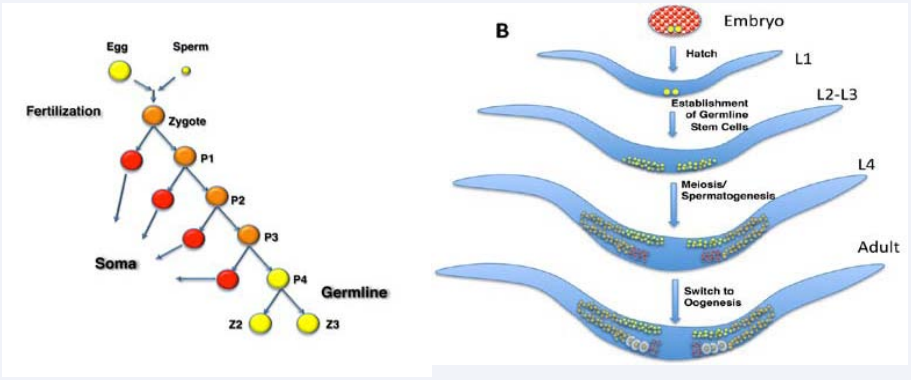


**Figure 1** Germline development in *C. elegans*. (A) Illustrates germline development in the embryo. The posterior daughter at each of the P1-P3 cell divisions illustrated gives rise to a somatic daughter and a germline precursor P cell. The P4 cell division yields germline committed cells, the Z2 and Z3 primordial germ cells, which do not divide further in the embryo. A yellow circle indicates committed germ cells, orange indicates non-committed cells, and red indicates somatic blastomeres. (B) Illustrates post-embryonic germline development in hermaphrodites. Z2 and Z3 remain quiescent until the embryo hatches and begins to feed. The germ cell divisions in early larval stages (L1 to late L2/early L3) create a germline stem cell pool (yellow circles). Meiosis (orang circles) begins in late L3 stage and in hermaphrodites the first cells to complete meiosis undergo spermatogenesis (red symbols). At the adult molt, the germline permanently switches to oogenesis (grey circles with yellow dots), with the stored sperm providing self-fertilization.

The Strome lab [32] recently showed that the embryonic germline function of PRC2 is to maintain the parental H3K27me patterns arriving with the gametes; i.e., to make sure the epigenetic memory of H3K27me established in the parental germ cells is accurately transmitted to the offspring's germline, where it is required to guide proper germ cell transcription [32]. Importantly, only the H3K27me patterns that arrive in the gamete chromatin is maintained in the embryonic germ cells; there appears to be little *de novo* addition until after proliferation is reactivated in larvae. For example, sperm chromatin inherited from fathers lacking PRC2 function lacked detectable H3K27me, and yet despite the presence of maternal PRC2 activity in the oocytes and zygote, the sperm chromatin stayed depleted of H3K27me throughout embryogenesis (Figure 3) [8]. This is remarkable, as it indicates that PRC2 activity in the embryonic germline is required solely for the maintenance of inherited, transgenerational H3K27me3. In the absence of PRC2-dependent maintenance, the pattern becomes diluted or possibly removed, and the post-embryonic germ cells attempt to proliferate and die.

Importantly, the requirement for PRC2 mediated repression in germ cells seems largely focused on the X chromosome. The X chromosome is enriched for H3K27me3 in adult germ cells [33]. Oddly, *mes* mutant males (which are XO haplotype) that inherit their X chromosome from the oocyte are always sterile (Figure 4A), yet a large majority of those that inherit their X from male sperm are fertile (Figure 4B). H3K9 demethylation is targeted to any unsynapsed chromosome during meiosis in either sex, and thus the X in XO males is always enriched in H3K9me2 [34,35]. This H3K9me enrichment in X chromatin is, like H3K27me3, maintained in sperm and inherited by the embryo [32]. Gaydos et al., showed that the enrichment of H3K9me on the unsynapsed X during spermatogenesis is required for the sperm-inherited fertility of XO *mes* males: *mes* male offspring from males lacking the H3K9 methyl transferases, MET-2 and SET-25, were sterile (Figure 4C). Unlike H3K27me3, however, maternally provided H3K9 methyl transferases can provide *de novo* addition of H3K9me to paternal chromosomes inherited from sperm lacking this mark in early embryos, suggesting different roles and modes of targeting for H3K9 methylation. Indeed, *C. elegans* employs a number of small RNA-directed processes that can direct H3K9 methylation to genomic loci to establish and maintain heritable gene silencing. These processes have been extensively reviewed elsewhere [36-38].

## Epigenetic Memory in the Germline II: H3K36 methylation

Another *mes* gene, *mes-4*, encodes an H3K36 methyl transferase that, like other MES factors, is maternally required and maternal supply is sufficient for fertility. Thus maternal supply, which becomes limited in the embryo to the Z2/Z3 germ cells, is also sufficient for the thousand descendants of these cells to become functional gametes. Despite the similarity in sterile phenotypes to that of PRC2 mutants, MES-4 appears to act in opposition to PRC2 by maintaining an epigenetic memory in genes that are active in germ cells, rather than repressed. In embryos, both MES-4 protein and the H3K36me it adds to chromatin are observed only in gene bodies of genes expressed in the parental germ cells [21,39]. Importantly, the H3K36me in gene bodies, a mark that normally correlates with active transcription, is independent of transcription of these genes in the embryo, since genes expressed only in germ cells lack detectable RNA Pol II and are inactive in early embryos. As with the PRC2 components, evidence suggests that MES-4 is unable to add H3K36me *de novo*, but rather maintains the patterns established in the parental germ cells and inherited through the gametes [21,39].

Another H3K36 methyl transferase, MET-1, appears to provide co-transcriptional H3K36me but is largely non-essential for fertility. Animals lacking MET-1 activity maintain fertility for many generations, although an increased frequency of sterility and sub-fertility can be detected in later generations [40]. Embryos lacking maternal MES-4 inherit H3K36me from gametes, due to transcription-dependent MET-1 activity in the parental germline, but this is not maintained beyond early divisions [21]. Conversely, MES-4 activities in *met-1* mutants maintain H3K36me

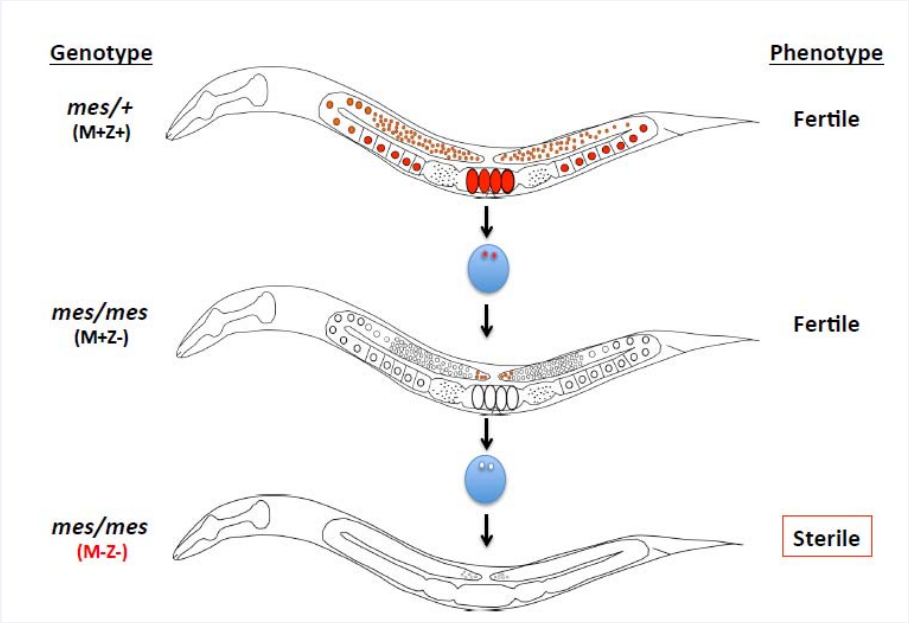


**Figure 2** Maternal Effect Sterility in *mes* Mutants. Homozygous (*mes/mes)* mutant offspring from heterozygous *(mes/+*) parents are fully fertile due to maternal loading (M+Z-) of the MES proteins into the oocyte (red) that are stabilized in the embryonic germline (red circles in embryo). These fertile animals cannot provide maternal MES proteins to their offspring (M-Z-), and the offspring produce only a few, necrotic gem cells during larval development and are completely sterile. MES function is probably only essential in the embryonic germline, as the ~1000 germ cell descendants from the M+Z- Z2/Z3 primordial germ cells lack MES activity. However, residual activity persisting in the germline stem cells has not been ruled out.

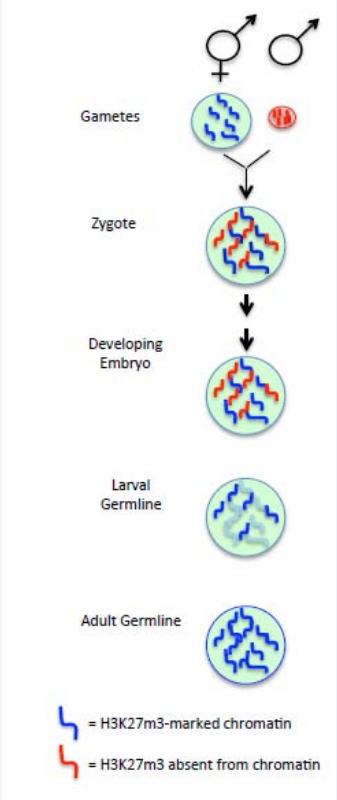


**Figure 3** PRC2-Dependent Memory of H3K27me3. When sperm from males lacking PRC2 function, and hence carrying chromosomes lacking H3K27me (red), are mated to wild type animals with normal PRC2 (blue), the offspring inherit two distinctly marked sets of chromosomes (zygote). Despite the presence of normal PRC2 activity in the embryo, H3K27me is not added *de* novo to the paternal chromosomes during embryogenesis. Interestingly, *de novo* addition *is* observed later in the larval germline, although the sites of this addition are not known.

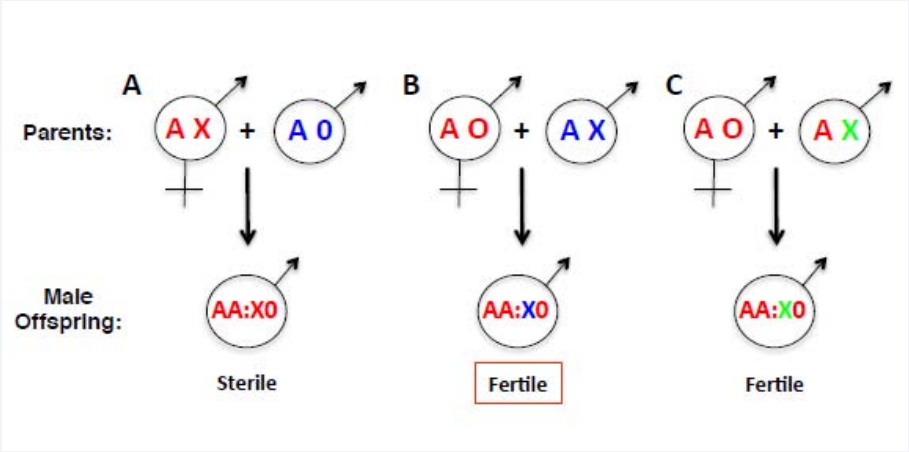


**Figure 4** Fertility Requires a Memory of Repression that is Mostly Focused on the X chromosome. A) Male offspring of fathers carrying autosomes marked by PRC2 (blue A) and mothers lacking PRC2 on both autosomes (red A) and the X chromosome (red X) are sterile. B) In contrast, if the X is inherited from a father with PRC2 activity (blue X), the male offspring are frequently fertile. C) The enrichment of H3K9me2 that occurs on the lone X in XO male germ cells (green X) is sufficient, even in the absence of H3K27me, to allow for the male offspring inheriting the H3K9me-enriched X to grow up fertile.

in germline-transcribed genes for many generations, despite the absence of transcription-dependent incorporation of this mark [21]. This raises the possibility that MES-4 is capable of faithfully maintaining the H3K36me patterns established by transcription when MET-1 activity was last present, many generations past. The generational increases in fertility defects observed in *met-1* mutants may be due to sporadic and accumulating mistakes from germlines relying solely on MES-4 maintenance, without transcription-dependent reiteration of the proper patterns at each generation.

The requirement for both PRC2-mediated repression and MES-4 dependent transcriptional memory suggests that a transgenerational balance between these two patterns must be maintained for normal germ cell development and function. Evidence suggests that MES-4, and presumably H3K36me, is antagonistic to PRC2 activities. In *mes-4* mutant germ cells, genes on the X chromosome show abnormally elevated expression; yet, neither MES-4 nor H3K36me are detected on the X in germ cells [39,41]. This counterintuitive result has been explained by an antagonistic model in which the normal depletion of MES-4 from the X chromosome is required for the observed enrichment of PRC2 on the X. In the absence of MES-4, loss of autosomal H3K36me allows for enhancement of autosomal H3K27me at the expense and loss of its enrichment on the X, thereby causing de-repression of X linked loci [39]. Support for this model has recently been provided by direct evidence of spreading of H3K27 me into loci whose normal enrichment for H3K36me is lost when MES-4 is depleted [42]. Thus maintenance of germline epigenetic memory is a balance between antagonistic memories of germline repression and germline transcription established and maintained in previous generations.

Importantly, this memory balance can also play out in somatic lineages, in which germline-restricted genes are normally repressed. Defects in conserved members of transcriptional repressor complexes, such as the Rb/LIN-35 retinoblastoma protein, lead to ectopic expression of genes whose expression is normally limited to the germline [43,44]. MES-4 and PRC2 functions are required for the ectopic activation of the "germline genes" in soma in *lin-35* mutants, indicating that correct epigenetic marking of these genes promotes expression if soma-specific repression mechanisms are defective. The *mes-4* gene is a direct target of *lin-35*- mediated repression in soma, which likely further counteracts MES-4's role in promoting expression of germline-expressed loci [45].

Oddly in some contexts MES-4 appears to be inhibitory to gene expression in germ cells. As mentioned above, the birth of Z2-Z3 is accompanied by transient RNA Pol II activation, as evidenced by the appearance of Pol II phosphoepitopes that correlate with transcription elongation [18]. The transient nature of this activation is not observed in *mes-4* mutants: Pol II phosphorylation persists, and this is accompanied by the appearance of MET-1 dependent H3K36me (normally transcription-associated) and pre-mature reappearance of H3K4me [21]. This apparently ectopic transcriptional activity, however, is not observed in PRC2/*mes-2* mutants [21]. In addition, the erasure of H3K4me that occurs in Z2-Z3 is normal in *mes-4* mutants, resulting in an embryonic germline that lacks normal levels of both H3K4 and H3K36 methylation. Whereas the lack of H3K4me, in the context of enrichment for H3K36me, may be inhibitory, the loss of both marks may be permissive for premature gene activation. Indeed, H3K36me is inhibitory to gene activation if present in promoters [46].

## Epigenetic Memory in the Germline III: H3K4 methylation

Another histone modification associated with transcription is H3K4me, which in *S. cerevesiae* is added to histone H3 during active transcription by the sole H3K4 methyl transferase, Set1 [17]. Set1 acts within the conserved COMPASS complex, for which many of the subunits are evolutionarily conserved [47,48]. In more complex eukaryotes, various H3K4 methyl transferases are found in a number of COMPASS-like complexes, including SET and MLL complexes, which have both unique and overlapping

subunits with COMPASS. Chief among these is WDR5, which is found in both COMPASS and MLL complexes, as well as others that appear not to contain methyl transferase activities [47]. The *C. elegans* genome encodes three WDR5 homologs of which only one, WDR-5.1, is required for normal H3K4 methylation [49-51]. Importantly, WDR-5.1 function appears to be important for the maintenance of H3K4me patterns in the absence of transcription [49]. Loss of WDR-5.1, or a subset of conserved COMPASS subunits, causes loss of H3K4 methylation in both early embryos and in the germline stem cell pool [49]. Mutations in *wdr-5.1* result in a "germline mortality" phenotype; i.e., there is an increasing frequency of sterile offspring at each successive generation after homozygosity for the mutation [49]. This is also observed in *set-2* mutants, which encodes an H3K4 methyl transferase that, from its overlapping phenotypes, is likely in a complex with WDR-5.1. Mutations in *wdr-5.1, set-2,* and another conserved COMPASS subunit, *rbbp-5*, all cause loss of H3K4me3 in GSCs [49,50]. In contrast, only loss of WDR-5.1 and RBBP-5 also cause decreases in H3K4me2 in germline stem cells, and loss or mutations in other conserved subunits (*ash-2, dpy-30, cfp-1*) have no significant effect on H3K4me in the GSCs, but exhibit dramatic decreases in both H3K4me2 and H3K4me3 in embryos [49,50]. These results are consistent with multiple H3K4 methyl transferases and complexes performing specific functions in different tissues.

The germline mortal phenotype of *wdr-5.1* and *set-2* mutants is most readily observed in animals grown at 25°C. Recent results from the Palladino lab [52] has shown that this is accompanied by an increased frequency of ectopic expression of genes normally only expressed in somatic lineages, decreased expression of germline-expressed genes, changes in cell phenotypes consistent with germline-soma trans-differentiation, and a decrease in germline-specific RNA-protein complexes called P-granules [52]. Oddly, the vast majority of genes exhibiting expression differences were up regulated in *set*-2 and *wdr-5.*1 mutants, and the overall level of the repressive modification H3K9me3 was also decreased, suggesting that loss of *set-2* causes derepression of genes; i.e., SET-2 activity is repressive. This is unexpected since H3K4 methylation is considered a mark of active chromatin and furthermore H3K4me and H3K9me are normally considered antagonistic. Indeed, loss of the H3K9 methyl transferase *met-2* causes increases in H3K4me and shows synergistic defects in the germline mortality phenotypes observed in animals that are also defective in the H3K4 demethylase, *spr-5/Lsd-1* [53].

Another phenotype observed in *set-2* mutants was a significant increase in H3K27me3, the PRC2-dependent mark of germline repression memory. Mammalian stem cells have been shown to have genes marked by "bivalent" chromatin; i.e., genes marked by both H3K27me and H3K4me [8]. Differentiation towards a specific lineage is accompanied by loss of one mark (e.g., loss of H3K4me in genes repressed in that lineage) and enrichment for the other (e.g., enrichment of H3K27me in the repressed genes). It is not known whether bivalent loci exist in *C. elegans*, but loss of *set-2* dependent maintenance of H3K4me in germline-expressed genes in the GSCs could lead to enrichment of H3K27me in these genes-- and their subsequent repression as was observed in this study [18].

The study by Kerr et al. [53], illustrates that an antagonistic balance between H3K9me and H3K4me, similar to the H3K36me/H3K27me antagonism, is important for heritable maintenance of germ cell function and fertility. As mentioned, mutation of SPR-5, the worm ortholog of the conserved H3K4 demethylase LSD1, results in a mortal germline defect [54]. The progressive sterility is accompanied by mis regulation of a number of genes normally expressed in sperm. Mutations in *met-2*, a homolog of the conserved SETDB1 H3K9 methyl transferase, results in similar phenotypes: increases in the frequency of sterile progeny and mis regulation of sperm-expressed loci with each successive generation [53]. The sperm-expressed genes, which normally exhibit MET-2-dependent H3K9me in embryos, show enriched H3K4me and ectopic expression in embryos in both mutants, indicating that at least for these loci, H3K4 and H3K9 methylation are mutually antagonistic. *spr-5; met-2* double mutants, in contrast to the single mutants that require many generations before maximal sterility, are sterile within a single generation. Thus the correct regulation of heritable patterns of H3K4me, presumably reinforced by opposing regulation by H3K9 me, is essential for proper development and function of the germline in the offspring. This is reminiscent of the maternal effect sterility observed in the *mes* mutants, although whereas simultaneous defects in both H3K9 and H3K4 methylation are required before offspring sterility is observed, loss of either H3K36me or H3K27me memory is sufficient.

## Epigenetic Memory in the Germline IV: H3K9 Methylation

MET-2 is also essential for another germline process, termed Meiotic Silencing. This process has been observed in many organisms from fungi to mammals and is characterized by the assembly of heterochromatin on unsynapsed chromosomal segments during meiosis [55]. In *C. elegans* meiotic silencing consists of enrichment for H3K9me2 in unsynapsed meiotic chromatin. In males, which are XO sex chromosome karyotype, the unsynapsed X is a natural target and indeed is highly enriched for H3K9me2 [34]. This enrichment is dependent on MET-2, as is H3K9me2 enrichment on any unsynapsed meiotic chromatin [56]. The function of this enrichment is not understood, but it has heritable consequences in the offspring: the X chromosome inherited from an XO parent shows delayed activation, and hence has been characterized as a form of imprinted X inactivation [34]. As mentioned above, another heritable consequence of the H3K9me2 enrichment on the X going through spermatogenesis is its suppression of the offspring sterility caused by a lack of maternal PRC2 function [32]. This indicates that X-linked gene repression, by any mechanism, is important for maintaining an epigenetics-based germline memory inherited from the parent. Unlike PRC2 repression, which must be retained as a presumed template for memory maintenance in the zygote, H3K9me is rapidly re-established in early stages, even on chromosomes coming in from parents lacking H3K9 methyl transferases [32]. As mentioned above, this efficient *de novo* re-establishment is probably guided by RNAi-related pathways that establish and maintain repression of their targeted loci.

## Epigenetic memory in the Germline V: A role for transcription

Chief among the mechanisms that are linked to the establishment of epigenetic information in the genome is RNA Polymerase II (PoI II) transcription. In addition to producing

RNA, the eukaryotic Pol II holoenzyme carries a number of other enzymatic activities, including histone methylases that target H3K4, H3K36, and H3K79 [57,58]. Phosphorylations of the C-terminal domain of the Pol II catalytic subunit, which define the phosphoepitopes described earlier, participate in the recruitment of histone modifying enzymes to the Pol II holoenzyme [59]. As a result, Pol II transit through a gene adds epigenetic information to that gene, providing a histone methylation "memory" that transcription had occurred in that gene in that tissue. In Drosophila somatic development [60,61], this memory is maintained by the Trithorax Group of proteins-activities that required to maintain lineage commitment [62], and include complexes that mediate H3K4 methylation [63,64]. The germ lineage likewise has such maintenance activities, and as a transgenerational lineage appears, at least in *C. elegans,* to maintain information imposed by transcription in the previous generation. The information that is maintained has to remain stable to exhibit heritability, despite the presumed "reprogramming" mechanisms that are observed, as well as the non-coding RNA-dependent genome surveillance mechanisms that seem to scan for and prohibit the novel or ectopic expression of genes.

Nevertheless, the germline transcription of trans genes in the adult germline has been shown to establish a heritable maintenance of H3K4me that correlates with increased somatic expression in subsequent generations [65]. Importantly, the persistence of H3K4me in the trans gene chromatin and enhanced somatic expression were observed even after germline expression of the trans gene was no longer detected [65]. This suggests that H3K4me marking at any time during germline development could create a persistent bias towards expression, and although this marking that may not override the repressive surveillance mechanisms operating in germ cells, may be sufficient to drive expression in the soma. This could explain the conserved "bivalent marking" of early developmental transcription factor genes observed in sperm and ES cells, characterized by promoter localized H3K4me and a broader and overlapping enrichment of H3K27me3 [11]. Any temporal recruitment of Pol II to these loci in germ cell precursor populations, even without productive elongation, could mark promoters with H3K4me. In the absence of elongation, there would be little or no addition of H3K36me and thus no antagonism against H3K27me, and there is little obvious antagonism between H3K4me and H3K27me. The maintenance modes of H3K4me and H3K27me addition would then act on these genes and heritably stabilize bivalent domains in germ cells and their pluripotent developmental intermediates.

In *C. elegans,* as in many other species, the germ cell lineage is not immediately set aside and temporally overlaps with cell populations that will contribute to somatic lineages. Each division of the P1, P2, and P3 germline precursor cells gives rise to a somatic blastomere and the next P cell**.** Defective PIE-1 inhibition of Pol II elongation causes the P2 cell to duplicate its somatic sister's fate, indicating that the instructions for somatic specification are present in the P cells, but prevented from engaging by PIE-1's inhibition of elongation (Figure 5) [18-20,66]. Indeed, maternal transcription factors driving somatic fates are equally distributed between P-cells and their somatic siblings, and phosphoepitopes corresponding to Pol II initiation are present (whereas those corresponding to elongation are absent) [18,67,68]. Therefore it is conceivable that some of the H3K4me maintained in the P-cells is due to recruitment of Pol II and associated enzymes, guided by maternal factors, to the promoters of somatic loci. PIE-1 inhibition of elongation would also prevent accumulation of elongation associated histone modifications, including H3K36me [21]. Thus there would be a clear demarcation of germline expressed versus potential soma restricted loci: the former would be marked by both H3K4me and H3K36me through memory maintenance, and the latter would only be marked by H3K4me. The genome-wide erasure of H3K4me observed in Z2-Z3 would then remove activating marks from all loci, but leave H3K36me to mark genes expressed in germ cells. Although there is no evidence for bivalent domains in *C. elegans*, it is conceivable that H3K27me could temporarily co-exist with H3K4me at somatic loci in the P-cells, although recruitment of Pol II to promoters by maternal factors would likely prevent its enrichment at promoters.

## CONCLUSION

*C. elegans* has proven to be an in valuable model system for the discovery of conserved aspects of developmental gene regulation, and continues to be an excellent experimental system for understanding epigenetic aspects of gene regulation. *C. elegans* short generation time has also made feasible analyses of transgenerational phenomena, which require analysis of the F3 generation and beyond. Numerous studies using *C. elegans* have shown transgenerational effects initiated by environmental exposures, including external stressors and nutritional limitations [69]. In this review we have attempted to summarize what has been discovered in this system about mechanisms that contribute to epigenetic memory and its generational stability in the germline. We have largely focused on histone methylation and its apparent role in ensuring normal germline regulation and fertility at each generation, since it is clear that histone H3 methylation plays a highly conserved role in modulating gene expression. Furthermore, results from studies using *C. elegans* have suggested the existence of antagonistic networks that establish and maintain epigenetic patterns in the germline, and hence reinforce heritable memory (Figure 6). It is also evident that non-coding RNA-based mechanisms, which contribute to genome surveillance, are essential for establishing and maintaining transgenerational gene repression in the germline, and that this mode of targeted repression has to be suppressed for expression of genes required for germline function. Thus gene repression and gene expression patterns in germ cells may be the result of transcription-dependent addition of histone modifications that are maintained across generations, but scrutinized by repressive genome surveillance mechanisms at each generation. The fidelity of the patterns is likely to be enforced by the stringent filter posed by fertility and embryonic viability: any incorrect pattern that significantly affects offspring viability or fertility will disappear. However, it is also possible that stochastic changes occurring in some genes that do not have

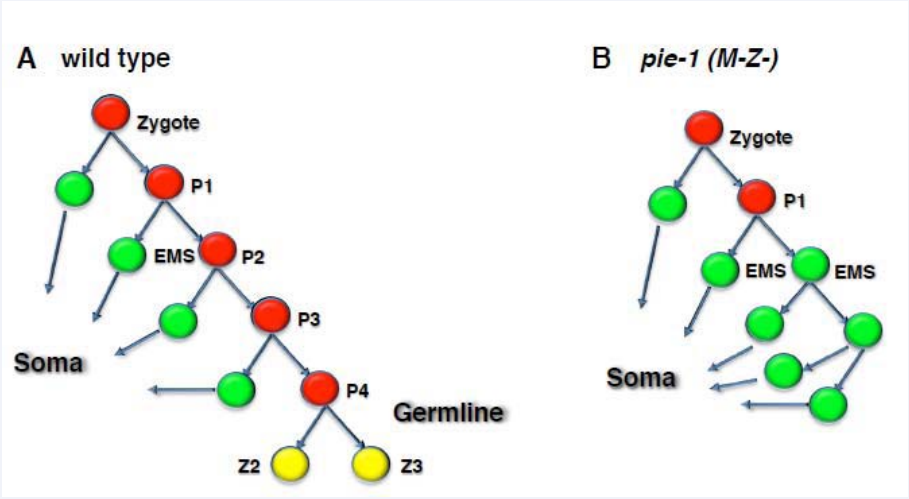


**Figure 5** Transcriptional Regulation in Embryonic Germ Cells. The P-lineage is normally transcriptionally inactive, and this repression requires maternal loading of the PIE-1 protein. At each P cell division in wild type embryos (A), PIE-1 is predominantly segregated to the posterior cell and RNA Pol II transcription is inhibited (red), whereas any PIE-1 ending up in the anterior cell is degraded and zygotic transcription begins (green). After P4 divides, PIE-1 degrades and active forms of Pol II are initially detected in Z2/Z3, but disappear shortly thereafter and remain low for the rest of embryogenesis (yellow). In the absence of both maternal and zygotic PIE-1 (B; M-Z-), activated RNA Pol II is ectopically detected in the P2 cell, leading to its conversion to a duplicate of its somatic sister (named EMS). This results in excessive development of the EMS descendant lineage, and concomitant loss of both the germline and all somatic descendants of P2. Thus the P cells appear to be programmed for somatic development, but are prevented from this fate by PIE-1-mediated Pol II repression.

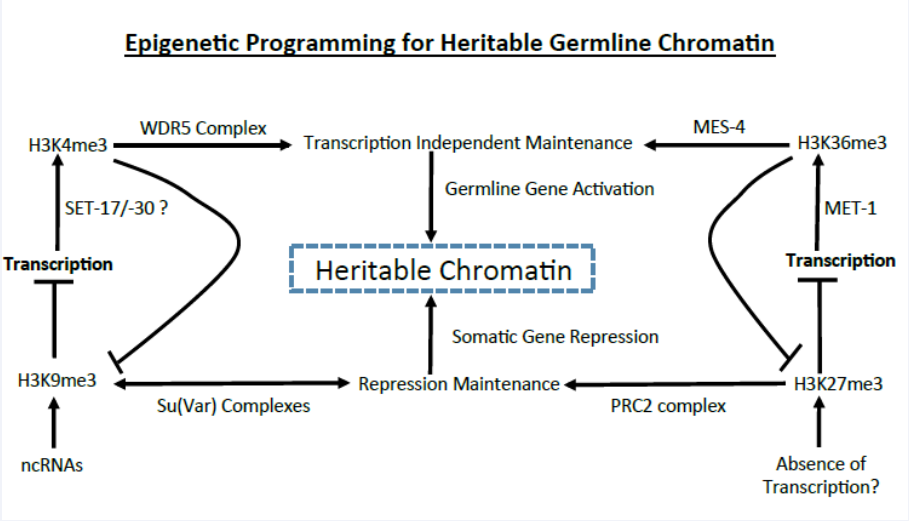


**Figure 6** Model for Antagonistic and Self-Reinforcing Epigenetic Network in *C. elegans* Germline Chromatin. The establishment of repressive chromatin (H3K9me3 and H3K27me3), by non-coding RNA or other mechanisms, is refractory to transcription and heritably maintained by Su (Var) (H3K9me3) and PRC2 (H3K27me3) complexes. In the germline this heritable (repressive) chromatin state is normally targeted to soma-specific genes. Transcription in the germline results in the establishment of H3K4me3 (SET-17 and/or SET-30) and H3K36me3 (MET-1)in the promoters and bodies, respectively, of both germline-restricted and ubiquitously expressed genes. This "transcription memory" is then heritably maintained in the germline by WDR5-containing complex (es) (H3K4me) and MES-4 (H3K36me). H3K4me and H3K36me in turn are antagonistic to the establishment and maintenance of heritable repressive chromatin, thus maintaining an epigenetic template that guides germline transcription in all generations. At each generation, the pattern is reinforced by the transcription dependent processes that are guided by the memory from the previous generation. Thus even transient ectopic activation or repression of genes in the germline has the potential to become stabilized and transgenerationally maintained.

significant effects on fertility could become incorporated into the memory system, which might play out in a developmental and/or post-developmental context. Therefore, following the concepts of Lamarckian evolution, environmental effects experienced by one generation could cause changes in germ cell epigenetic information that become heritably established, and maintained by an antagonistic and self-perpetuating network in subsequent generations.

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