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# Transmission of chromatin states across generations in *C. elegans*

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ABSTRACT

Epigenetic inheritance refers to the transmission of phenotypes across generations without affecting the genomic DNA sequence. Even though it has been documented in many species in fungi, animals and plants, the mechanisms underlying epigenetic inheritance are not fully uncovered. Epialleles, the heritable units of epigenetic information, can take the form of several biomolecules, including histones and their post-translational modifications (PTMs). Here, we review the recent advances in the understanding of the transmission of histone variants and histone PTM patterns across generations in *C. elegans*. We provide a general overview of the intergenerational and transgenerational inheritance of histone PTMs and their modifiers and discuss the interplay among different histone PTMs. We also evaluate soma-germ line communication and its impact on the inheritance of epigenetic traits.

## 1. Introduction

Milestone experiments led by Oswald Avery and colleagues as well as Alfred Hershey and Martha Chase showed that DNA, not protein, is the main biomolecule carrying heritable information [1,2]. Today, however, we know that there are mechanisms beyond DNA that can pass information to the next generations. Epigenetic factors contribute to the transmission of information that affects many traits such as longevity, fertility, stress response and disease susceptibility [3–7]. Epigenetic inheritance affects gene expression without changing DNA sequence and may persist even after the event that triggers the change in gene expression is no longer present. Epigenetic traits can be inherited from one cell cycle to the next, but can also be passed on to the next generations. Based on the persistence of these traits, epigenetic inheritance is classified into two main categories: (i) intergenerational epigenetic inheritance (IEI), in which the traits only persist for one generation, and (ii) transgenerational epigenetic inheritance (TEI), in which the traits remain observable for several generations (**Fig. 1**). For a recent comprehensive review on IEI and TEI, see [8]. The underlying molecular mechanisms of epigenetic transmission are not completely understood, but histone PTMs and histone variants play a key role in this process.

Histones are positively charged proteins that constitute nucleosomes, the basic structural unit of chromatin. Nucleosomes organize the genome into higher-order structures and are involved in many DNA-related processes including the regulation of gene expression, DNA replication, DNA repair and chromosome segregation. Typically, histone proteins consist of two domains: a histone fold domain (HFD) and an unstructured N-terminal tail. The HFD enables the heterotypic interactions among histones to form the nucleosome core that wraps DNA, while the N-terminal tails are the main substrate regions for PTMs.

The most common PTMs of histone N-terminal tails are methylation and acetylation of lysine residues, and phosphorylation of serine, threonine, and tyrosine residues. The methyl and acetyl groups are deposited by histone methyltransferases (HMTs) and histone acetyltransferases (HATs), hereafter collectively called writers [9]. The histone writers' activities can be reversed by demethylases and deacetylases that thus act as erasers. The modification of histone tails often results in the alteration of the nucleosomal interaction partners and access to the DNA. PTMs can be recognized by specific reader proteins that can recruit additional transcriptional regulators or histone writers and erasers. Through these actions, they can regulate transcription and are associated with active or repressed gene expression. For instance, methylation of H3K9 and H3K27 is mainly associated with transcriptionally silent regions (called heterochromatin), while methylation of H3K4 and H3K36 is correlated with transcriptionally active regions (called euchromatin). In this review, writers, erasers, and readers are collectively called histone modifiers.

Histone variants are histone proteins that can replace replication-dependent histones to alter nucleosome function [10–12]. Histone variants are generally expressed outside of S phase and are involved in a

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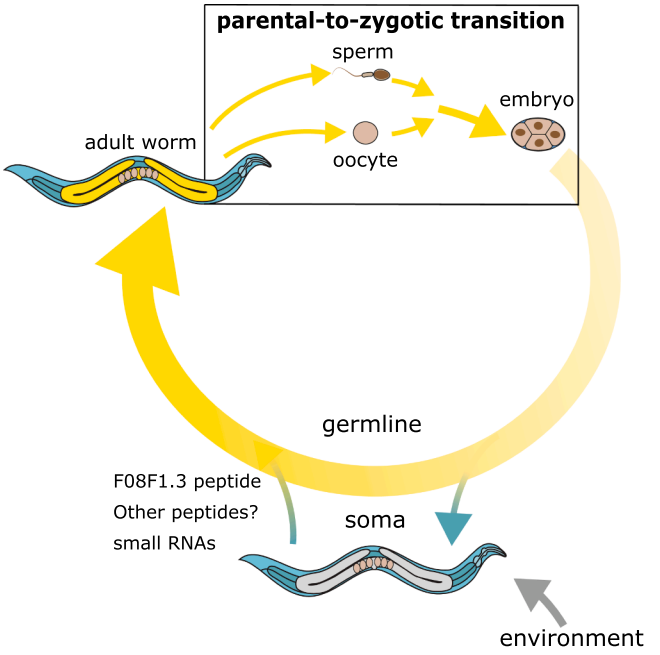


**Fig. 1.** Schematic view of epigenetic inheritance in *C. elegans*. The germ line forms a continuum (shown in yellow) along which chromatin states can be inherited. Chromatin states can be transmitted from the parental generation to the zygote (top). The distributions of histone variants and histone PTMs frequently undergo reprogramming during the production of germ cells or upon fertilization. Chromatin states can also be maintained from one cell cycle to the next and throughout development into the adult germ line (yellow circle). This maintenance might be perturbed in response to environmental cues that act on the soma and can signal to the germ line to induce epigenetic changes (bottom). These newly established states can be inherited for one (intergenerational inheritance) or several (transgenerational inheritance) generations despite the removal of the conditions that initially triggered the changes.

wide range of cellular functions. As they can also be post-translationally modified, the functions of histone variants and PTMs in epigenetic inheritance are overlapping.

The genomic distribution patterns of histone PTMs or histone variants can be maintained across cell cycles and generations [13]. A critical question is how this maintenance occurs through DNA replication, cell division and sexual reproduction. A current model proposes the following coordinated continuum steps: (i) modified histones are evicted during DNA replication; (ii) those evicted parental histones are incorporated at the same genomic regions, but randomly distributed between the two daughter chromatids and thus being diluted; (iii) empty spaces on the chromatin are replenished with new histones by histone chaperones to restore the original nucleosome density; (iv) newly incorporated histones are modified or replaced by histone variants based on the nearby inherited nucleosomes, through the action of histone modifiers [14–18].

One of the main challenges in epigenetic inheritance is maintaining traits through sexual reproduction, as chromatin undergoes significant remodeling during the different stages of meiosis and upon fertilization in the zygote [19,20]. Moreover, zygotic transcription is not detectable in very early stages, and many chromatin modifiers are maternally contributed to enable the establishment of the desired chromatin modifications immediately upon fertilization. The maternal-to-zygotic transition is therefore a key moment in IEI (Fig. 2).

*C. elegans* has emerged as a convenient model organisms to study the inheritance of histone PTMs. Self-fertilization, the invariant cell lineage during development, a short generation time, a large brood size and evolutionarily conserved epigenetic mechanisms enable the study of epigenetic inheritance at cellular and generational levels. The *C. elegans*

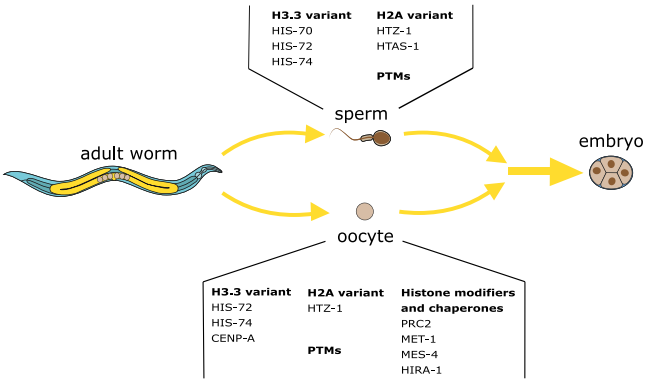


**Fig. 2.** Transmitted histone PTMs, variants and modifiers from parents to progenies through sperm and oocytes. Both sperm and oocytes carry different histone variants and PTMs from parents to the offspring, while histone modifiers are mainly deposited through oocytes.

germ line is organized in an assembly line fashion, harboring a stem cell niche in the distal tip and mature oocytes or sperm in the proximal gonad, enabling the observation of germ cell chromatin during mitosis and different meiosis stages within the same animal. *C. elegans* populations mostly consist of self-fertilizing hermaphrodites, but males arise at a low frequency through non-disjunction of the X chromosome. Hermaphrodites produce both oocytes and sperm and contain two X chromosomes per diploid cell (XX), while males produce only sperm and contain one X chromosome per diploid cell (X0). In the germ line of both sexes, the X chromosomes are extensively silenced by repressive histone PTMs (Fig. 3). Global suppression of X-linked gene expression by heterochromatin is essential for germline development and fertility [21]. This makes the *C. elegans* X chromosome an insightful model to detect the alterations in the genomic distribution of histone PTMs.

In *C. elegans*, sperm chromatin is mainly organized using histones and histone variants, not protamines as is the case in vertebrates (Fig. 2) [22]. A number of histone PTMs are therefore detectable in sperm, in part explaining how the epigenetic inheritance of histones and their PTMs can occur through the paternal germ line. However, sperm chromatin undergoes substantial reorganization upon fertilization, and maternally provided chromatin proteins appear in the male pronucleus already before pronuclear fusion [22,23].

Here, we focus on recent advances in our understanding of epigenetic inheritance that is based on histone variants and histone PTMs in

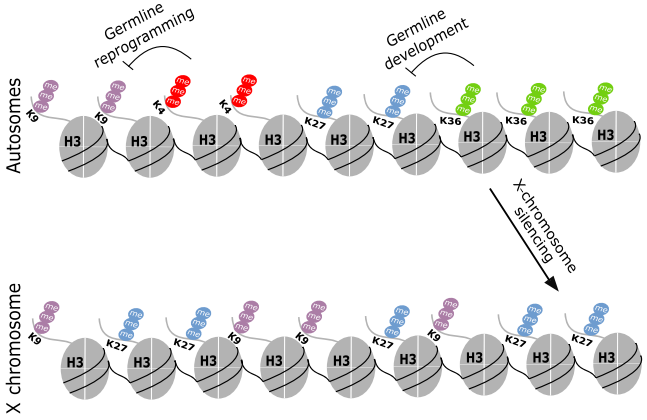


**Fig. 3.** Cooperation and competition of histone PTMs in germline reprogramming and development. On autosomes (top), H3K4me3 (euchromatic, red) influences the distribution of H3K9me3 (heterochromatic, purple), and H3K36me3 (euchromatic, green) limits the spreading of H3K27me3 (heterochromatic, blue). The X chromosome (bottom) is enriched in heterochromatic PTMs, and the autosomal H3K36me3 influences the distribution of H3K27me3.

*C. elegans*. Many of the histone variants and modifications that we discuss here are conserved among eukaryotes, although the precise function in epigenetic inheritance might differ between taxa. There is a significant body of research investigating the role of small RNAs in epigenetic inheritance that we only briefly comment on, and that is reviewed in detail elsewhere [24–26]. We first evaluate the roles of histone variants in the inheritance of chromatin states. We also provide a general overview of the transmission of histone PTMs and their regulators across generations. Later, we discuss examples of epigenetic inheritance of histone PTMs and their crosstalk. Finally, we highlight recent examples of how the environment can influence epigenetic inheritance through germ line reprogramming.

## 2. Histone variants in epigenetic inheritance

Most taxa, including *C. elegans*, encode replication-independent histone variants that can substitute the canonical histones in nucleosomes to carry out specialized functions. Histone variants are often distributed in specific genomic patterns, and are present in oocytes or mature sperm, making them good candidates for carrying epigenetic information from one generation to the next (Fig. 2).

One major variant of histone H3 is H3.3, which has a vital role in a variety of processes such as stress response, fertility, embryonic stem cell differentiation, neuron plasticity, centromere maintenance and epigenetic reprogramming following somatic nuclear transfer [27–29]. H3.3 differs from canonical H3 by four amino acid residues (31, 87, 89 and 90). While the phosphorylation of serine 31 can affect the methylation of lysine 27, amino acids 87–90 mediate the recognition by the H3.3-specific histone chaperones, and thus influence the localization and maintenance of H3.3 [30].

The *C. elegans* genome encodes five identifiable homologs of H3.3, and each homolog has a distinct expression patterns [27,31]. Among them, HIS-70, HIS-72 and HIS-74 are expressed in germ cells and are transmitted to next generation [27]. HIS-72 and HIS-74 are inherited through the maternal and paternal gametes, while HIS-70 is transmitted only through sperm (Fig. 2) [27,31]. Immediately following fertilization, maternal HIS-72 is incorporated into paternal chromatin [31]. The parentally inherited H3.3 is present on autosomes but not on the transcriptionally inactive X chromosome, implying a transcription-linked inheritance of H3.3 [31].

In addition to the H3.3 homologues mentioned above, the histone H2A variants HTZ-1 and HTAS-1 are present on sperm chromatin (Fig. 2) [22,27]. HTZ-1 is inherited from both parents and removed from chromatin after fertilization, while HTAS-1 is only transmitted through sperm and remains bound to the parental chromatin until the 4-cell stage (Fig. 2) [22]. The different modes of inheritance of HTZ-1 and HTAS-1 suggest that they are maintained or removed by distinct mechanisms [22]. This may be in part achieved through ubiquitin-mediated proteasomal degradation, as paternal H2AK48ub and H2AK63ub are removed from chromatin and degraded during late spermatogenesis and after fertilization [22].

While the presence of these histone variants in gametes suggests that they are involved in transmitting patterns from one generation to the next, the cellular processes for which this transmission is important have not been identified. Loss of H3.3 is tolerated in *C. elegans*, with phenotypes that are not obviously linked to the maternal-to-zygotic transition. However, loss of the H3.3-specific histone chaperone HIRA-1 results in late-onset phenotypes such as small size, pale coloring and defecation defects in the offspring of the first generation of the homozygous HIRA-1 null mutants. Interestingly, these late-onset phenotypes are phenocopied upon mitochondrial perturbations, suggesting that at least part of the defects is driven by chronic mitochondrial stress. These results are consistent with the finding that some mitochondrial mutants are long-lived, and that this extension of lifespan depends on H3.3 [29,32]. Maternally provided HIRA-1 is sufficient to suppress the late-onset defects of *hira-1* null progenies, highlighting the importance of H3.3 assembly in the inheriting generation (Fig. 2) [32]. It is conceivable that maternally deposited HIRA-1 can persist into adults in sufficient amounts to rescue these late-onset defects. Alternatively, maternally deposited HIRA-1 may be required to establish chromatin states in embryos that are subsequently maintained into adult animals [32].

CENP-A, another variant of histone H3, defines centromeric chromatin and is essential for chromosome segregation. The inheritance of centromeres is an epigenetic process that varies in detail between organisms [33]. *C. elegans* chromosomes are holocentric, with CENP-A distributed along the length of the chromosomes [34]. It is present in all mitotic cells, but shows a discontinued distribution in the germ line, where it is removed at the mitosis-to-meiosis transition, and reappears on chromatin at diplotene of prophase I. Likely as a consequence of holocentricity, CENP-A is not required for the meiotic chromosome segregations in *C. elegans* [35]. It is nevertheless present in the proximal germ line and transmitted to embryos through the oocyte, but is absent in sperm (Fig. 2). Our lab recently showed that the N-terminal tail of CENP-A is essential for setting centromere identity in the proximal germ line, and that this identity is heritable for one generation. In the absence of the N-terminal tail, cell divisions and development are unaffected for one generation, but centromere identity cannot be reset in the adult germ line, leading to severe segregation defects and embryonic lethality in the offspring [36]. De novo loading and maintenance of CENP-A chromatin both involve the loading factor KNL-2 and the histone chaperone LIN-53 [36–38]. Moreover, centromere establishment on worm artificial chromosomes depends on HAT-1-mediated acetylation of H3K9 and H4 [39,40].

Accumulating findings obtained from cytological, genetic, genomic and evolutionary studies indicate that histone variants and the factors acting in their assembly can contribute to epigenetic inheritance. Many of them are germline-specific but dynamic in early embryos, suggesting that their main role is in IEI. The expression of histone variants is actively regulated during gametogenesis and fertilization in *C. elegans*, and perturbation of this regulation can lead to detrimental defects [32,36,41]. It will be of great interest to determine how histone variants shape the chromatin states in the zygote. It will also be important to identify the histone PTMs that decorate the inherited histone variants. Recent evolutionary studies led to the identification of novel H2A and H2B variants in mammals [42,43]. As is the case for many histone variants in *C. elegans*, these variants show germline-specific expression [42,43]. These findings suggest that the role of histone variants in IEI might be universal, and that they might be involved in genetic conflicts during reproduction [43].

## 3. Histone PTMs in epigenetic inheritance

Regulation of gene expression ensures that genes needed for cell function and tissue development are transcribed by RNA polymerases, while other genes are kept in a repressed state. This regulation needs to be dynamic, so that it can vary over time and between cell types. Histone PTMs are ideal to fulfill such a regulatory role, as their genomic distribution patterns can be inherited and preserved, but also remodeled during the life cycle of an organism (Fig. 1). For some histone PTMs, there are multiple histone modifiers that may have distinct functions in either the initial establishment of the modified chromatin state or the maintenance of this state during development or to the next generations. Consistent with the key roles of histone PTMs in early development, many of them are templated by parentally inherited histone PTMs and parentally provided modifiers. For example, MES-4 and MET-1 (H3K36me2/3 writers), as well as the PRC2 complex (H3K27me3 writer), are maternally supplied [44–46].

### 3.1. Heterochromatic histone PTMs

Repressive histone PTMs (also known as heterochromatic PTMs), which include H3K9me, H3K27me and H3K23me, are associated with

epialleles that are heritable across generations [47]. H3K9me is mainly driven by two HMTs, MET-2 and SET-25 [48,49]. MET-2 deposits H3K9me2/3 while SET-25 catalyzes H3K9me3 [49–52]. H3K9me2 and H3K9me3 seem to have distinct binding sites and roles in the genome [53]. H3K9me2 is enriched on DNA transposons and telomeres whereas H3K9me3 is particularly associated with retrotransposon families [53]. Immunofluorescence experiments revealed that they also exhibit distinct localization patterns in the germ line [52]. The levels of H3K9me2 are low at the pachytene stage, increase in diplotene and then decrease in diakinesis, while H3K9me3 levels remain constant throughout the gonad [52]. H3K9me3 is only present in oocytes but not sperm, suggesting that it is not paternally inherited to the next generations [54]. While the accumulation of H3K9me2 in *wdr-5* (a component of the COMPASS complex, discussed in section 5.2) mutants leads to a transgenerational extension of the lifespan of animals, changes in H3K9me3 levels do not affect longevity [5].

MET-2 limits the duration of transgenerational inheritance of small RNAs, and resets the zygote's epigenetic ground state. In *met-2* mutants, aberrant endo-siRNAs accumulate over generations, eventually leading to a "mortal germline" (*mrt* phenotype), a multigenerational phenotype where animals become progressively sterile over several generations [55–59]. However, silencing of a piRNA-targeted reporter gene and the establishment of nuclear RNAi-mediated silencing requires SET-25 (and SET-32, see below) but not MET-2 [51,56,60–63]. Despite the fact that SET-25 and MET-2 deposit methyl groups on the same histone residue (H3K9), they are involved in distinct transgenerational processes which may be partly linked to the different genomic elements they act on [4,5,64].

The H3K9 HMTs required for the maintenance of heterochromatin might work less efficiently at elevated temperatures. A multi-copy heterochromatic reporter was upregulated in subsequent generations of worms whose mothers were kept at 25 °C [65]. The misexpression of the reporter was attributed to the reduction of H3K9me3, and wild type levels were restored only 15 generations after the heat shock in a SET-25-dependent manner [65,66]. Of note, a single reporter copy did not show this memory effect, implying that the inheritance depends on the repetitive nature of the reporter transgene [65,66].

As summarized above, several lines of experiments uncovered transgenerational phenotypes associated with H3K9me. Despite the presence of H3K9me in the germ line and the transgenerational phenotypes with misregulated H3K9me, the inheritance of the PTM itself between generations has not been experimentally validated. It is possible that H3K9 HMTs or small RNAs act as the inherited molecules, and that they can establish H3K9me patterns in the inheriting generations [67].

The most abundant histone PTM found in embryos is H3K27me3 [68]. H3K27me3 is present on all chromosomes, but is enriched on the X chromosome, where it is important for gene silencing to maintain germline identity (**Fig. 3**) [23,44,69–72]. H3K27me3 is both maternally and paternally inherited, and the deposited levels of H3K27me3 are sufficient to support embryogenesis [23,73]. H3K27me is mediated by the PRC2 complex (consisting of the subunits MES-2, MES-3, MES-6) and marks genes that are spatio-temporally repressed, but retain the potential for expression. PRC2 acts both as a reader and writer of H3K27me. It is only maternally supplied and is required for maintaining H3K27me3 levels in later stages of development [23,73,74]. Upon fertilization, PRC2 requires pre-existing H3K27me3 on maternal and paternal chromatin to maintain H3K27me3 distribution patterns [23,73]. Therefore, H3K27me3 itself transmits the short-term memory of gene repression, while PRC2 is required for the maintenance of repressed chromatin and thus promotes the long-term memory during development [23].

Defective transmission of H3K27me3 and PRC2 results in defects in germline development and the maintenance of cellular identities, and in a maternal-effect sterile (*mes*) phenotype [23,44,73]. Fertilization with H3K27me3-deficient sperm results in a derepression of somatic genes, especially neuronal genes, predominantly from the paternal chromosomes in the germline [73]. These defects likely arise because PRC2 in *C. elegans* is unable to deposit untemplated H3K27me and is antagonized by H3K36me (discussed in section 5.1) (**Fig. 3**), which prevents the re-establishment of silencing on chromatin that lacks inherited H3K27me3 [44]. H3K27me3 and PRC2 are intergenerationally inherited; however, due to the lack of tools that can bypass the *mes* phenotype, a potential transgenerational inheritance of H3K27me3 could not be assessed.

Methylation of H3K23 is another PTM associated with heterochromatin that has been implicated in epigenetic inheritance [75]. H3K23 modifications are ubiquitously present in embryonic somatic cells. While H3K23me1 appears diffuse in the nuclei, H3K23me2/3 are found in bright and discrete foci, suggesting that H3K23me1 might have different roles from H3K23me2/3 [68]. H3K23me1 and H3K23me3 are present in PGCs at similar levels in embryos, but H3K23me2 appears reduced in PGCs both in embryos and in freshly hatched larvae, suggesting that it might undergo remodeling during development [68].

SET-32 methylates H3K23, but not any other tested lysine residue on H3 based on in vitro radioactive labeling assays, and the levels of methylated H3K23 are reduced genome-wide in *set-32* mutants [75]. It is currently unknown whether SET-32 is inherited between generations, but the distribution patterns of H3K23me3 directed by nuclear RNAi are transgenerationally inherited in a SET-32-dependent manner [75]. Initially, SET-32 was described as an HMT for H3K9, but the total levels of H3K9me3 were not reduced in *set-32* mutants, and methylation levels of H3 by SET-32 were unchanged in vitro upon H3K9L mutation, revealing that H3K9 is not the target of SET-32 [51,75]. The changes of methylated H3K9 in *set-32* mutants are perhaps an indirect consequence of a reduction in H3K23me [60,62]. H3K23me3 levels are also slightly reduced in *met-2;set-25* (H3K9 HMTs) double knock-out strains, suggesting a synergistic relationship between the two PTMs [75]. Total H3K27me3 levels increase in *set-32* mutants [51], and H3K23me2 is enriched in regions decorated with H3K27me3 or H3K9me3 [68]. These associations suggest that H3K23me may indirectly affect the inheritance of heterochromatin states by altering the levels of H3K9me3 and H3K27me3.

Formation and maintenance of heterochromatin are critical for organizing the genome and regulating gene expression. Defects in heterochromatin formation and maintenance affect numerous processes such as germline development and immortality, nuclear chromosome localization, genome stability, and RNAi-induced gene silencing [49,50,53,58,61,63,76,77]. The histone modifiers that maintain heterochromatin across generations intersect with different small RNA pathways in *C. elegans*. The piRNA- and nuclear RNAi-mediated silencing largely, but not solely, depend on H3K9me3 [60,78]. Additionally, SET-25 and SET-32 are required for the establishment of RNAi silencing, but dispensable for the maintenance of the RNAi effect in subsequent generations [51,63]. Heterochromatic PTMs are enriched at nuclear RNAi targets and epigenetically inherited for several generations [75]. Consistently, HRDE-1, an essential component of the germline-specific nuclear RNAi complex, is required for the induction of heterochromatic PTMs [63,75,79].

### 3.2. Euchromatic histone PTMs

While the epigenetic inheritance of heterochromatic PTMs is well established, it has been questioned whether this is also the case for euchromatic PTMs [80]. However, there is accumulating evidence that euchromatic PTMs contribute to phenotypes that are epigenetically inherited across generations [3,81].

One prominent PTM for active transcription is H3K36me. In *C. elegans*, H3K36me2/3 is enriched on autosomes and depleted from large portions of the X chromosome (**Fig. 3**) [82]. Methylation of H3K36 is mediated by MET-1 and MES-4 [45,82]. However, MET-1 and MES-4 show differences in their spatiotemporal expression patterns and

functions in germ lines and embryos [83]. While MET-1 catalyzes H3K36me3, MES-4 can catalyze both H3K36me2 and H3K36me3. Maternal MES-4 associates with paternal chromosomes bearing H3K36me3 after fertilization, suggesting that MES-4 can also act as a reader, and that pre-existing H3K36me3 is required for its writing activity [45,82]. Therefore, MES-4 was proposed to intergenerationally transmit the memory of gene expression [83]. MET-1 levels rapidly diminish during early embryogenesis, and then increase coincident with zygotic genome activation, indicating that MET-1 activity is co-transcriptional [45,46]. Overall, these findings suggest a model where MET-1 can deposit H3K36me3 in parental germ cells during transcription, and MES-4 can maintain the distribution patterns of H3K36me3 and the expression memory of germline-specific genes in the inheriting generation [46,83,84]. Later, MET-1 can again mark the transcribed genes with H3K36me3 during development [45].

H3K4me is another well-studied euchromatic PTM. Different levels of H3K4me (mono-, di- or tri-) are associated with distinct loci. H3K4me1/2 are enriched at promoters and enhancers, while H3K4me3 is mostly found at promoter regions of active genes. H3K4 is methylated by the conserved SET1/COMPASS complex. In *C. elegans*, this complex contains SET-2 (H3K4me3 writer), ASH-2 and WDR-5.1 [85,86]. WDR-5.1 is the most closely related worm homologue of human WDR5, and WDR-5 is therefore used synonymously with WDR-5.1 for the rest of the review. SET-2 and ASH-2 are required for global H3K4me in the germ line and in embryos. Additionally, ASH-2 has a role in soma-to-germ line transfer of epigenetic information (discussed in detail in Section 6). Consistent with the differential roles of the members of the SET1/COMPASS complex, each can differentially contribute to gene expression in the germ line [87].

During transcription, the SET1/COMPASS complex (H3K4me3 writer) interacts with elongating RNA polymerase II and promotes H3K4me. Thus, H3K4me could transmit a memory of transcription [83,88]. However, immunostaining experiments indicated that H3K4me2 is present in oocytes and sperm, but H3K4me2/3 are erased in PGCs in embryos and then reappear in larval and adult germ line, thus questioning the heritability of H3K4me [46,89,90]. Interestingly, the reappearance coincides with the initiation of transcription, as these PTMs are not visible in starved (transcriptionally incompetent) hatchlings [89]. If the H3K4me reprogramming is impaired and H3K4me2 accumulates, meiotic defects, transgenerational increased longevity and a progressive decline in fertility are observed, suggesting that both loss and accumulation of H3K4me have detrimental consequences [48,81,91,92].

Mutations in SET1/COMPASS complex components can cause epigenetically inherited defects such as genome instability, transgenerational loss of germline identity, and a progressive decline in fertility and transgenerational longevity [3,87,93–95]. Since H3K4me2/3 undergoes extensive reprogramming, it is not clear whether these defects are based on impaired inheritance of histone PTMs, or whether the SET1/COMPASS complex can affect heritable phenotypes independent of its HMT activity. A progressive decline in fertility can be the consequence of loss of transcriptional memory and has recently been proposed to occur in two step process in *set-2* (H3K4me3 writer) mutants: (i) a priming step in early generations where loss of SET-2 leads to misexpression of genes that has a limited impact on fertility and (ii) a more widespread misexpression in later generations leading to a decline in fertility and eventually sterility [94]. Transgenerational transcriptomic analysis of *set-2* mutants indicated that the total number of misregulated genes in later generations was approximately three times higher than in early generations [94]. The majority of downregulated genes are germline-specific and are required for germline maintenance, including meiosis factors and P-granule components, while the upregulated genes are soma-specific or X-linked and promote cell differentiation, for example LIN-15B, the C/EBP homolog CEBP-1, GLP-1/Notch signaling genes and TGF-β pathway components [94]. A large proportion of the upregulated X-linked genes in *set-2* mutant are also misregulated in the absence of PRC2 (H3K27me3 writer) or MES-4 (H3K36me3 writer), suggesting that PRC2/MES-4 and SET-2 act in parallel pathways to silence the X chromosome [82,94]. The aforementioned defects are partly explained by the de-silencing of the X chromosome [87,94]. Since the X chromosomes are depleted from H3K4me in the germ line, loss of H3K4 HMTs may have an indirect effect on the transcription on the X chromosomes **(**Fig. 3**)** [31,82,90,94].

## 4. The interplay between histone PTMs

Combinations of different histone PTMs can influence the local chromatin conformation and activity. The histone PTMs co-existing on the same nucleosomes were recently assessed by an unbiased mass-spectroscopy analysis [68]. Both positive and negative correlations of different histone PTMs play significant roles in epigenetic inheritance, which may be particularly relevant during the maternal-to-zygotic transition **(**Fig. 2**)**. For example, multivalent nucleosomes are modified by both heterochromatic and euchromatic PTMs. Such multivalent nucleosomes, containing H3K36me3, H3K27me3 and low levels of H3K4me3, are found at spermatogenesis-specific genes in *C. elegans* sperm [22,45,74]. The histone PTMs are maintained upon fertilization, but the euchromatic PTMs are subsequently lost during early embryogenesis [74].

### 4.1. Heterochromatin vs H3K36me3

There is increasing evidence that H3K9me3 and H3K27me3 have overlapping roles in germline maintenance. For example, the double knockout (but not any of the single knockouts) of *cec-3* and *cec-6*, readers for H3K9me3 and H3K27me3, respectively, result in a *mrt* phenotype, implying that H3K27me3 and H3K9me3 can act redundantly [6].

The suppressed state of X chromosomes required for germline maintenance is mainly mediated by PRC2 (H3K27me3 writer) and inherited through oocytes and sperm [23]. PRC2 null males are still largely fertile, while PRC2 null hermaphrodites are maternal effect sterile (*mes*), suggesting that alternative histone PTMs may play a role in the repression of the paternally inherited X chromosome. Genetic analysis revealed that H3K9me3 is partly sufficient to suppress the paternal X chromosome in the absence of PRC2 [23]. Consistently, much of the H3K9me3-marked chromatin coincides with the distribution of H3K27me3 [47] **(**Fig. 3**)**.

*mes-4* (H3K36me3 writer) mutants show upregulation of the same X-linked genes that are also upregulated in PRC2 null animals, resulting in a similar *mes* phenotype [82,84]. Combined mutations of *mes-4* and PRC2 synergize the fertility defects, suggesting that H3K36me3 and H3K27me3 act on the same targets [44]. Given the fact that they have opposing roles in gene expression regulation, observing parallel phenotypes is surprising. Indeed, H3K27me3 and H3K36me3 occupy mutually exclusive domains, implying that MES-4 and H3K36me3 may dictate the localization of PRC2 and H3K27me3 in a maternal effect fashion **(**Fig. 3**)** [44,82,96]. The cooperative and antagonistic relationship between H3K36me3 and H3K27me3 is necessary for proper development and cell fate [44,96]. The *mes* phenotypes of PRC2 and *mes-4* mutants suggest that if this relationship is disturbed, proper silencing patterns are preserved for one generation, but result in germline proliferation defects of the next.

### 4.2. H3K4me2/3 vs H3K9me2/3

Mutations in *spr-5* (H3K4me3 demethylase) result in the accumulation of H3K4me2 and a progressive decline in fertility, which can be exacerbated upon the loss of H3K9me2 [48,58]. These results reveal that H3K4me2 and H3K9me2/3 have opposing roles in germline reprogramming **(**Fig. 3**)** [48,58]. Additionally, the accumulation of H3K9me2 upon the perturbation of H3K4me suggests that H3K4me2/3 limits the spreading of H3K9me2/3 [5,48].

SET1/COMPASS complex (H3K4me3 writer) mutants show transgenerational longevity phenotypes, even though global H3K4me3 levels in the wild type progenies of SET1/COMPASS-impaired parents are not decreased [3]. This implies that the transgenerational inheritance of lifespan is either associated with heritable local changes of H3K4me3 at certain loci, or the inheritance of another molecule. Recently, the accumulation of H3K9me2 was suggested to be one of the underlying causes for the transgenerationally inherited longevity of *wdr-5* mutants (a member of SET1/COMPASS) [5,58]. SPR-5 (H3K4me3 demethylase)/MET-2 (H3K9 writer)-mediated differentiation of soma from germ cells occurs through the suppression of maternally deposited MES-4 (H3K36 writer) that is important to maintain the expression of germline genes [97,98].

Together, these studies reveal that cells need to maintain an intricate balance between euchromatin and heterochromatin (Fig. 3). Since some of these states are heritable, mutation of histone modifiers often does not lead to immediate phenotypes, but to defects that are only revealed in subsequent generations.

## 5. The transmission of environment-induced epialleles through soma-to-germline communication

Environmental conditions can induce changes in gene regulation and in the distribution of histone PTMs. Such changes can be heritable if they occur in the germ line, as epigenetic information typically is transferred from germ line to soma. However, in the case of environmental cues, the soma may signal to the germ line to establish and maintain epialleles (Fig. 1) [95,99]. Environment-induced epialleles can be inherited either maternally or paternally, implying that the transmission of specific epigenetic information rather than a simple maternal contribution is required [4,65,99]. One example of such soma-to-germline signaling is observed upon high glucose treatment, which in both hermaphrodites and males leads to a reduction in fecundity and elevated H3K4me3 levels, while subsequent generations grown under normal conditions are oxidative stress-resistant [100]. This transgenerational oxidative stress resistance requires the SET1/COMPASS complex [100]. Interestingly, the elevated levels of H3K4me3 in the high glucose level-exposed parental generation are not inherited to the F1 and F2 generations [100], raising the question how the memory of high glucose treatment is passed on to the offspring.

Mild stress exposure of the parental generation can lead to increased stress resistance and an extended lifespan for at least two subsequent generations [4,95,99]. This inherited stress resistance depends on the presence of WDR-5, a member of the SET1/COMPASS complex, in the inheriting generations [99]. Contrarily, intestine-specific depletion of *ash-2,* another member of the SET1/COMPASS complex, increases the oxidative stress response, which is dependent on the germline expression of RBR-2 (H3K4me3 demethylase) and SPR-5 (H3K4me3 demethylase) [95]. This increased stress resistance lasts for two generations, indicative of an epigenetic memory of the stress exposure through the germ line. Tissue-specific RNAi and knock-out studies seem to be a powerful approach to identify the initiating and responding genes/-pathways of soma-induced epigenetic inheritance [95,99]. Such genetic screens might allow us to gain a deeper understanding of the communication between soma and germ line.

Upon alterations in the environmental conditions, changing the epigenome might be more practical and advantageous compared to the acquisition of genetic mutations, as changing the genomic sequence can be inefficient and slow. In contrast, epiallelic adaptations by histone PTMs can occur relatively quickly and in a reversible manner. How the signaling between soma and germ line occurs remains largely elusive. Recently, a signaling peptide (F08F1.3) was shown to be released from the intestine to the germ line during the oxidative stress response (Fig. 1) [95]. Additionally, small RNA pathways are involved in the inheritance of epialleles resulting from environmental stimuli [55,101,102], making small RNAs candidate molecules for transmitting signals between tissues (Fig. 1) [64,95].

## 6. Systemic interaction of natural gene variation

The growing collection of wild type *C. elegans* strains is turning out to be a powerful resource for identifying genes involved in complex heritable traits [103,104]. For example, an allele of the *set-24* gene isolated from a wild type strain can lead to a *mrt* phenotype at 25 °C when crossed into other wild isolates or the N2 reference strain [103]. Even though the biological role of SET-24 is unknown, it contains a SET domain, suggesting that it could be involved in the methylation of histone N-terminal tails [103]. Crossing different wild type strains generates multi-allelic mosaics that may enable the identification of additional genes involved in epigenetic inheritance [104]. The advances in genetics, genomics and proteomics techniques are making it feasible to identify the causal variants underlying such observed heritable phenotypes.

## 7. Conclusion

Chromatin regulation is essential for the survival and reproduction of individuals. During early embryonic development, the distribution patterns of histone PTMs are not always established de novo. Rather, progenies receive marked histones and their modifiers as well as histone chaperones from the parental germ lines to instruct their epigenome (Fig. 2) [32]. A number of recent studies in *C. elegans* revealed that both the maternal and the paternal contribution of histone PTMs are essential [23,27,65,73,74,99]. Maternal and paternal chromatin was proposed to carry distinct histone PTMs into the early embryo and undergo distinct histone remodeling events before and after fertilization [22]. Several lines of experiments revealed crosstalk among different histone PTMs. In some cases, the dynamic crosstalk hampers the identification of the target of histone writers and erasers, making a careful parallel analysis of the interacting PTMs essential.

In this review, we focused on the inheritance of histone variants and histone PTMs in *C. elegans*. However, there are also other mechanisms acting in epigenetic inheritance. Small RNAs mediate the setting and inheritance of gene expression states across several generations. In some instances they function by themselves, but growing evidence indicates a causal link between the formation of heterochromatin and small RNA pathways [55,75,76,79]. Several overlapping phenotypes were detected as a consequence of the defects in the inheritance of histone PTMs and mutants affecting small RNA pathways [60]. Furthermore, genes regulating the formation and maintenance of heterochromatin are required for the inheritance of small RNA-mediated silencing [53,76]. The exact mechanism underlying the function of heterochromatin in RNAi remains to be described; however, heterochromatin clearly contributes to the epigenetic inheritance of silencing induced by RNAi [62,75,76,78,79].

The examples we discuss focus on the methylation and acetylation of histones at the most studied sites, and the deposition of histone variants. Additionally, the N-terminal tails of histones can undergo other types of post-translational modification including ubiquitination and phosphorylation, as well as methylation of H3K14, H3K79 and H4K20 [68,105–108]. However, our understanding of whether these PTMs are epigenetically inherited is currently lacking. Future studies will add to our understanding of how inherited chromatin states are passed on between generations, and how heritable phenotypes are linked to the different histone PTMs and their interactions. It will be exciting to understand in more detail what changes at genomic and transcriptomic levels occur in the inheriting generations to adapt to the new epigenomic states.

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### *Declarations of interest*

The authors declare no conflict of interest.

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