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***Caenorhabditis elegans* as an emerging model system in environmental epigenetics**

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Abstract

The roundworm *Caenorhabditis elegans* has been an established model organism for the study of genetics and developmental biology, including studies of transcriptional regulation, since the 1970s. This model organism has continued to be used as a classical model system as the field of transcriptional regulation has expanded to include scientific advances in epigenetics and chromatin biology. In the last several decades, *C. elegans* has emerged as a powerful model for environmental toxicology, as well, particularly for the study of chemical genotoxicity. Here, we outline the utility and applicability of *C. elegans* as a powerful model organism for mechanistic studies of environmental influences on the epigenome. Our goal in this review is to inform the field of environmental epigenetics of the strengths and limitations of the well-established *C. elegans* model organism as an emerging model for medium throughput, *in vivo* exploration of the role of exogenous chemical stimuli in transcriptional regulation, developmental epigenetic reprogramming, and epigenetic memory and inheritance. As the field of environmental epigenetics matures, and research begins to map mechanisms underlying observed associations, new toolkits and model systems, particularly manipulable, scalable *in vivo* systems that accurately model human transcriptional regulatory circuits, will provide an essential experimental bridge between *in vitro* biochemical experiments and mammalian model systems.

I. Introduction

The roundworm *Caenorhabditis elegans* has been an established model organism for the study of genetics and developmental biology, including studies of transcriptional regulation, since the 1970s (Corsi et al. 2015). This model organism has continued to be used as a classical

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model system as the field of transcriptional regulation has expanded to include scientific advances in epigenetics and chromatin biology (Padilla et al. 2014; Corsi et al. 2015). In the last several decades, *C. elegans* has emerged as a powerful model for environmental toxicology, as well, particularly for the study of chemical genotoxicity (Leung et al. 2008). To date, the nascent field of environmental epigenetics, which explores the impacts of environmental insults on epigenetic patterns and machinery, has largely focused on rodent models or epidemiological studies in human populations. Here, we outline the utility and applicability of *C. elegans* as a powerful model organism for mechanistic studies of environmental influences on the epigenome. First, we describe the strengths and limitations of *C. elegans* as a model system for environmental toxicology and genotoxicity. Next, we expand to include specific strengths and limitations of this model system for mechanistic epigenetics research and provide a concise literature summary of notable epigenetics findings in *C. elegans*. Finally, we summarize the current literature on environmental epigenetics research in *C. elegans* and highlight high impact, high interest research foci in this subfield that are ideally suited to the nematode system. Our goal in this review is to inform the field of environmental epigenetics of the strengths of the well-established *C. elegans* model organism as an emerging model for medium throughput, *in vivo* exploration of the role of exogenous chemical stimuli on transcriptional regulation, developmental epigenetic reprogramming, and epigenetic memory and inheritance.

II. *Caenorhabditis elegans* as a model system in environmental toxicology and genotoxicity

History of *C. elegans* in environmental toxicology and genotoxicology

C. elegans was first described at the turn of the 20th century as *Rhabditis elegans* by French librarian and biologist Emile Maupas who recounted coming across the nematode species twice near the city of Algier (Algeria) (Maupas 1900). He proceeded to publish a remarkably detailed treatise on nematodes in which he described *C. elegans*' anatomy and development and conducted some early experiments on the nematode, such as inducing a starvation response. Victor Nigon and Ellsworth Dougherty studied *C. elegans* and refined the culture methods necessary to the worms' propagation in the laboratory in the early 1940s, but *C. elegans* did not become an established model organism until Sydney Brenner's work was published in the 1970s. Brenner aspired to molecularize developmental biology and to dissect the genetic contributions to development and phenotype based on specific features, including invariant developmental pattern, transparency, rapid life cycle, and ease of culture (Nigon and Felix 2017). Initially of interest primarily to geneticists and developmental biologists, basic research in the nematode nonetheless laid important groundwork for toxicological research, for example by characterizing DNA damage (Hartman and Herman 1982) and stress responses (Fatt and Dougherty 1963; Blum and Fridovich 1983) at phenotypic and genetic levels. Phil Williams and colleagues first developed *C. elegans* as a toxicological model organism (Williams and Dusenbery 1987; Williams and Dusenbery 1988; Williams and Dusenbery 1990a; Williams and Dusenbery 1990b). Jonathan Freedman and colleagues subsequently pioneered heavy metal response characterization (Slice et al. 1990; Freedman et al. 1993), toxicogenomic analysis (Cui et al. 2007), and medium-throughput toxicity testing (Boyd et al. 2007). Richard Nass established

the use of *C. elegans* for chemical-induced neurodegeneration (Nass et al. 2001; Nass et al. 2002). The number of groups doing such work has grown rapidly in recent years, and *C. elegans* is now a well-characterized model for human and ecological toxicity and genotoxicity studies (Leung et al. 2008; Steinberg et al. 2008; Boyd et al. 2010; Helmcke et al. 2010; Allard et al. 2013; Choi et al. 2014; Meyer and Williams 2014; Ferreira and Allard 2015; Tejeda-Benitez and Olivero-Verbel 2016; Honnen 2017; Hunt 2017; Lenz et al. 2017; Maurer et al. 2017).

Strengths of *C. elegans* as a model organism for molecular toxicology

Many of the same characteristics that make *C. elegans* a valuable model for developmental biology make it a powerful model for toxicology studies (Fig. 1). These characteristics include:

1. A short life cycle (3–4 day generation time) that allows efficient evaluation of age- or developmental stage-specific and transgenerational effects, and enables experiments that are otherwise complex, time-consuming, and expensive in mammalian models (Corsi et al. 2015).
2. A well-studied and annotated genome (*C. elegans* was the first metazoan genome to be sequenced (Consortium 1998)), in which 60–80% of the genes have human homologues (Kaletta and Hengartner 2006). In addition, there is a growing collection of wild strains, catalogued and curated by the *Caenorhabditis elegans* Natural Diversity Resource (CeNDR; www.elegansvariation.org), that is a powerful resource for identifying genetic components leading to natural trait variation (Cook et al. 2017). For example, a wild strain, named CB4856, was discovered in Hawaii in the 1970s but only recently well characterized. This strain has been leveraged, together with the common laboratory strain N2, to create recombinant inbred lines for mapping of genetic loci contributing to naturally occurring phenotypic variation (Andersen et al. 2015). Molecular toxicology studies with these strains have begun (Zdravljek et al. 2017).
3. Exceptionally powerful genetic tools that facilitate the study of gene-environment interactions (multiple gene knockout consortia (Consortium 2012a) and a “million mutation” project resulting in multiple mutations in every gene in the genome (Thompson et al. 2013), plus two whole-genome RNA interference (RNAi) libraries (Kamath and Ahringer 2003; Rual et al. 2004)).
4. Widely available transgenic strains (Antoshechkin and Sternberg 2007), growing university-based research resources for analysis of tissue-specific gene expression, including promoter-GFP fusions and other tools (<http://www.gfpworm.org/>), as well powerful CRISPR-dCas9 genome editing techniques, that permit *in vivo* analysis of specific phenotypes, including gene expression, morphology, and more.
5. A transparent body and invariant somatic developmental pattern and anatomy (Sulston and Horvitz 1977; Kimble and Hirsh 1979; Sulston et al. 1983) that facilitate analysis of altered development.

6. *C. elegans* is an important and predictive tool in reducing the use of vertebrates in molecular toxicology research, which is an important ethical consideration. For example, a recent publication from the National Toxicology Program provided strong empirical support for the human health relevance of *C. elegans* toxicity testing (Boyd et al. 2015). In chemical developmental toxicity screens using EPA's ToxCast™ Phase I and II libraries (high throughput toxicity screening of nearly 1,000 chemicals), *C. elegans* results were nearly as predictive of toxicity in the classic rat model as another mammalian model (rabbit) (Boyd et al. 2015). Multiple studies from different research groups have found that there is a strong correspondence between *C. elegans* and higher eukaryotes in rank-order acute toxicity of chemicals, based on strong conservation of molecular targets and signaling pathways relevant to chemical toxicity (reviewed in (Hunt 2017)).

The natural habitat of *C. elegans* is decomposing organic matter, typically at the soil surface (Schulenburg and Felix 2017), but exposures can be carried out either on solid support (typically, agar) or in liquid. Liquid exposures facilitate medium- and high-throughput assays, for example of neurotoxicity (Boyd et al. 2009), behavioral toxicity (Boyd et al. 2007), nanoparticle toxicity (Maurer et al. 2015; Hanna et al. 2016), reproduction (Boyd et al. 2010; Allard et al. 2013), inducers of mitochondrial hsp-60 (Rauthan and Pilon 2015), and SKN-1 (NRF-2 homolog) inhibitors (Leung et al. 2013).

The power of this model for biomedical research, and fundamental biological discoveries first made in *C. elegans*, are reviewed by Corsi et al. (Corsi et al. 2015). Notable cross-species biological phenomena including apoptosis (Hedgecock et al. 1983), miRNA (Lee et al. 1993), and RNA interference (Fire et al. 1998) have been discovered in *C. elegans*, resulting in some cases in Nobel prizes (Horvitz 2003; Sulston 2003). Relatedly, a strength of this model is the large body of knowledge related to stress response pathways. While relatively few *C. elegans* researchers explicitly study toxicology, a large proportion study stress responses, in particular with respect to lifespan regulation, which is extensively examined in the nematode. There is an extensive literature on stress responses in *C. elegans*; selected examples of well-studied stress response pathways particularly relevant to environmental toxicology are the DNA damage response (Rieckher et al. 2017), apoptosis (Conradt and Xue 2005; Bailly and Gartner 2013), autophagy (Melendez and Levine 2009; Zhang et al. 2015), antioxidant defenses (Brys et al. 2007; Blackwell et al. 2015), nuclear receptor signaling (Antebi 2015), mitochondrial stress responses (Haynes and Ron 2010), and heavy metal responses (Caito et al. 2012).

Limitations of *C. elegans* as a model organism for molecular toxicology

C. elegans has emerged as a powerful model for the study of molecular and cellular-level toxicity, as well as for understanding *in vivo* effects such as cell-to-cell interactions and developmental dynamics. However, as for all model organisms, there are a variety of limitations; in the case of *C. elegans*, many are the inherent counterparts of the simplicity that makes the worm a powerful model. We recently reviewed genetic, biochemical and physiological differences between the worm and mammals relevant to toxicity (Maurer et al. 2017) – here, we will highlight a few key differences. Perhaps most obviously, *C. elegans*

lack many of the anatomical features of mammals, from cell types such as blood cells to organ systems such as lungs. Worms have no blood transport, no blood brain barrier, and, despite effective chemical metabolism, no human-like first-pass metabolism in the liver or blood filtration in the kidney. Signaling pathways and subcellular phenomena important in those cells and processes may often be studied through isolation and characterization of function and chemical concentrations in specific organelles, but ecology of the intact organ as it is present in a person cannot be mimicked. In addition, although acute chemical toxicity in *C. elegans* is often similar to higher eukaryotes, as noted under *Strengths* in this section, species differences in toxicokinetic and toxicodynamic parameters have not been systematically assessed, although a few are well known. For example, *C. elegans* are covered by a relatively impermeable cuticle layer, or with an eggshell prior to hatching, and possess robust mechanisms for excreting toxic chemical compounds, both characteristics that can complicate or alter apparent sensitivity to chemicals. The routes of exposure of the nematode are also poorly understood although the heightened sensitivity to chemicals in cuticle mutants (Kishimoto et al. 2017) suggest that exposure is likely transdermal as well as through ingestion at least in some cases. However, growing evidence suggests that internal concentrations of pollutants and drugs are similar in worms and mammalian models, even in cases of high exposure concentrations, and nematode strains with compromised cuticles can be used to reduce this concern (Zheng et al. 2013; Luz et al. 2016; Xiong et al. 2017). In addition, genetic and biochemical differences exist in regulation and catalytic activity of metabolic enzymes, including cytochrome P450 and other metabolic enzymes and transporters. Based on human and *C. elegans* sequence homology data, *C. elegans* has a larger complement of cytochrome P450 genes that does not completely overlap with the human complement, and apparently excludes specific toxicologically important P450s (Leung et al. 2010). However, it is possible that human genes without direct sequence homology in *C. elegans* may have uncharacterized functional orthologues for these genes that can be informative. The presence of functional orthologues can be detected using metabolic diagnostic tests that assess metabolism of a known chemical reaction substrate. Metabolic diagnostics to assess presence or absence of specific P450 orthologues are rarely performed; we are aware of only one study that confirmed the absence of *Cyp1a1*, which bioactivates the commonly-studied contaminant benzo[a]pyrene (Leung et al. 2010). If both sequence-based and functional orthologues are absent, use of *C. elegans* enables isolation of effects of specific human P450s, through development of transgenic strains that express human P450s but avoid the potential bias that accompanies suppression of endogenous P450s. Cytochrome P450 genes are not the only gene class that is expanded in *C. elegans*, as compared to humans; others that are clearly relevant to toxicology include nuclear hormone receptor (*nhr*) and Fbox genes, which should be strong considerations for experiments with chemicals known to require or interact with members of these gene classes. As with P450 genes, such differences may be intentionally employed as part of experimental design. For example, although humans have both innate and adaptive immune systems, because *C. elegans* lacks an adaptive immune system, we were able to isolate effects of inorganic mercury and methylmercury on the innate immune system (Wyatt et al. 2017). Similarly, although humans rapidly methylate inorganic arsenic after absorption and often have mixtures of parent chemical and metabolites in circulation, because *C. elegans* lacks an arsenic methyltransferase, we were able to isolate the effect of inorganic arsenic on

mitochondrial function (Luz et al. 2016). Despite these differences, with expanded use of worms in environmental toxicology, worm toxicokinetics have been described for a growing list of pollutants, including nanoparticles (Yang et al. 2017), cisplatin (Crone et al. 2015), chlorpyrifos (Roh et al. 2016), and phenanthrene (Spann et al. 2015). In addition to species-specific differences, experimental culture conditions are an important consideration in molecular toxicology experiments in *C. elegans*. *C. elegans* can be cultured in liquid or solid media, under a wide range of conditions, many of which directly impact organismal metabolism, behavior, and stress resistance phenotypes (Szewczyk et al. 2003; Lithgow et al. 2017).

III. *Caenorhabditis elegans* as a model system for epigenetics

History of *C. elegans* as a model for the study of epigenetic regulation

C. elegans is uniquely well suited to the study of transcriptional regulation, cell fate specification and differentiation by transcription programs, due to enhancers located close to initiation sites, body transparency, and functional knockdowns via RNAi (Corsi et al. 2015). These and other characteristics recommend *C. elegans* as an exceptional model organism for mechanistic studies of epigenetic responses to environmental stimuli.

Strengths of *C. elegans* as a model organism for epigenetics

Several aspects of *C. elegans* that make it suitable as a model for environmental toxicology research also make it an advantageous model for epigenetics research (Fig. 1), albeit not always for the same reasons. For example, as noted in Section II, *C. elegans* has a relatively short life cycle, which makes it an excellent model for exploration of whether and how different developmental environments can influence epigenetic patterning over the life-course and over several generations (Corsi et al. 2015). As also noted in Section II, *C. elegans* is a powerful genetic model with extensive genetic, transcriptional and translational toolkits that permit functional follow-up of epigenetic questions (Corsi et al. 2015). *C. elegans*' fully mapped and invariant cell lineage ensures analysis of the same number of cells in each whole animal or cell type-specific experiment, removing tissue heterogeneity problems that are common in epigenetics experiments in rodent models (Sulston and Horvitz 1977; Kimble and Hirsh 1979; Sulston 2003).

In addition, there are several characteristics of the nematode that make it specifically suited for epigenetics research (Fig. 1). These include:

1. Epigenetic and epigenomic processes can be visualized in real-time at the single-cell level in the developing organism in real time using advanced microscopy techniques. The nematode's invariant embryonic cell lineage was originally described using manual methods, including visual confirmation in embryos using differential interference contrast (DIC) microscopy. These techniques later included DIC time-lapse microscopy and manual lineage tracking of individual embryos. More recently, embryos expressing histone::fluorescent fusion proteins were used to track nuclear divisions using confocal fluorescent time-lapse microscopy (Garcia et al. 2012; Hibshman et al. 2016), which enables analysis of many more embryos over much longer time windows as compared to manual

methods. Newer semi-automated nuclear tracking algorithms are capable of single cell-resolution phenotyping. Single cell-resolution phenotyping enables accurate characterization of even subtle, quantitative, or pleiotropic phenotypes. For example, pleiotropic regulators, such as chromatin remodeling proteins, may be tracked to assess a wide range of context-specific functions (Kruger et al. 2015). Transparent bodies allow for easy visualization of chromatin structure and gene expression. In particular, this feature enables real-time, *in vivo* single molecule studies, as well as real-time ordering of biochemical steps in transcriptional regulatory cascades (Wang and Sherwood 2011).

2. The general conservation of gene regulation, yet absence of long-range enhancer control of gene expression (Consortium 2012b), simplifies mechanistic experiments and enables development and confirmation of simple mechanisms that will inform the more complex transcriptional regulation of mammalian genes.
3. Germ cells are easily accessible and therefore easy to study. Studies of germline epigenetics or meiotic heritability of epigenetic alterations are feasible (Corsi et al. 2015), as are studies that experimentally clarify the relative role of sperm or oocytes to heritable phenotypes (Klosin et al. 2017).
4. There is a significant and growing body of work on comparative epigenetics in model organisms *Drosophila melanogaster* and *C. elegans*. The modENCODE (Model Organism Encyclopedia of DNA Elements) Consortium, complementary to the ENCODE consortium in human cells, has mapped functional elements in these two organisms genome-wide, including mRNA transcripts, post-translational chromatin modifications, regulatory factor binding sites, and origins of DNA replication in developmentally staged animals, tissues, and cell culture (Brown and Celniker 2015). Systematic comparisons of genomic distribution and regulatory functions of epigenetic marks in model organisms with existing data in mammalian cell culture systems have demonstrated that, despite differences in genome organization among species, relationships among chromatin states, transcription, and co-transcriptional RNA processing are highly conserved. Publicly available datasets, for example, include developmental time courses of RNA sequencing in *C. elegans*, with 15-minute resolution across embryogenesis (Brown and Celniker 2015).

C. elegans is a biologically appropriate model for mechanistic studies of mammalian transcriptional regulation

Epigenetic regulatory systems in *C. elegans* are highly conserved across phyla (Hodgkin 1994; Gaudet and McGhee 2010). Mechanisms of transcriptional control are well described in *C. elegans*, including posttranslational histone tail modifications, chromatin remodeling proteins, and non-coding RNA (Hodgkin 1994; Gaudet and McGhee 2010). Although *C. elegans* largely does not utilize the covalent DNA modification 5-methyl cytosine (Simpson et al. 1986; Hu et al. 2015), CpG islands appear extant in *C. elegans* (Chen et al. 2014); this finding may be due to an evolutionarily recent loss of this mark. Some invertebrates, including nematode species closely related to *C. elegans*, do contain low levels of methyl

cytosine, or “mosaic methylation” (Feng et al. 2010; Capuano et al. 2014; Gao et al. 2014). However, it has been suggested that invertebrate methyl cytosine likely functions to suppress ectopic promoter activity in genic regions of moderately transcribed genes, rather than as a primary regulator of gene expression, as in vertebrates (Branciamore et al. 2010; Chen et al. 2014). Therefore, invertebrates are likely not appropriate models for interrogating the functional significance of genome-wide DNA methylation in humans, although they may be useful models for specific functions of genic DNA methylation. Nonetheless, invertebrate systems are powerful experimental systems for determining other modes of epigenetic control of gene expression and chromatin structure, among other functions (Padilla et al. 2014). Several seminal discoveries in epigenetic control of gene regulation have been made in invertebrate systems, including discovery of the *Polycomb* and *Trithorax* chromatin remodeling protein families in *D. melanogaster* (Capuano et al. 2014) and the first identification and functional characterization of a histone demethylase in *C. elegans* (Greer et al. 2014).

Worms facilitate experiments that are complex, time-consuming, and expensive to perform in mammalian models, including developmental time courses in isogenic populations, induction of environmental insults, and functional validation with a powerful genetic toolkit (Brown and Celniker 2015). Invertebrate models are ideal for delineation of stepwise mechanisms that can then be further tested in a vertebrate model, such as mice. In addition, DNA methylation is unlikely to be the first responder to environmental insults, rather functioning to stabilize new gene expression changes induced by histone tail modifications (Samson et al. 2014; Shibata et al. 2014). Therefore, if early events following toxicant exposure are the primary endpoints, *C. elegans* is well suited to answer these questions.

Limitations of *C. elegans* as a model system for epigenetics

For research intended to inform human health outcomes, the primary limitations of *C. elegans* as a model system are its lack of DNA methylation, a mark that likely has greater functional relevance in mammals than in nematodes, and its lack of organ systems, including tissues and cell types that may be specific chemical targets and may experience tissue-specific epigenetic effects, for extension of epigenetic changes to specific human health endpoints (Corsi et al. 2015). Even in studies primarily aimed at delineating transcriptional regulatory circuits, the primary limitation of *C. elegans* is also one of its strengths; *C. elegans* does not use long range transcriptional regulation (Corsi et al. 2015), which recommends it a simplified model for mapping mechanisms but also negates the possibility of studying the role of long range regulation relevant to other animal species.

Epigenetics research in *C. elegans*

a. Covalent DNA modifications—DNA methylation refers to covalent modification of a nucleotide base with a methyl group, including 5-methylcytosine (5mC) or 6-methyladenine (6mA). Most commonly used non-mammalian model organisms lack genome-wide DNA methylation (including yeast, fruit fly, and worm) (Feng et al. 2010; Sarda et al. 2012). However, genome project studies indicate that DNA methylation is an evolutionarily ancient regulatory mechanism and that loss of DNA methylation is derived and generally lineage-restricted (Sarda et al. 2012; Capuano et al. 2014; Gao et al. 2014). Comparisons between

phyla or species on the use of DNA methylation as a regulatory feature are often based on the presence or absence of DNA methylation proteins, including maintenance methyltransferases *dnmt1* (general function) and *dnmt2* (highly specific function (Raddatz et al. 2013), as well as most evolutionarily wide-spread (Ponger and Li 2005)), *de novo* methyltransferase *dnmt3*, and methyl binding protein *mbd2* (Gutierrez and Sommer 2004). Species with functional DNA methylation typically encode some or all of these enzymes and species without DNA methylation seem to have lost these enzymes from their genomes.

5-methyl cytosine: Invertebrates from diverse phyla generally display mosaic methylation, or regions of methylated DNA interspersed with regions of unmethylated sequence (Suzuki et al. 2007). Mosaic methylation likely represents the ancestral state of the chordate genome, and genome-wide DNA methylation is thought to be limited to vertebrates (Albalat et al. 2012). In invertebrates, evolutionarily conserved genes, including “housekeeping” genes, tend to be methylated, and highly expressed genes tend to be unmethylated, supporting the hypothesis that CpG methylation suppresses spurious initiation of transcription in moderately, but not highly, transcribed genes (Suzuki et al. 2007). Invertebrates with low or undetectable CpG methylation, including *D. melanogaster* and *C. elegans*, appear to be exceptions (Suzuki et al. 2007). The genome of *D. melanogaster* was long considered unmethylated, but recent reports support region-specific methylation at specific stages of embryogenesis (Gutierrez and Sommer 2004). A single study has reported the presence of low level DNA methylation in *C. elegans* (Hu et al. 2015), although this report has not yet been replicated. Genome-wide vertebrate CpG methylation is thought to serve several functions, including transcriptional control and suppression of repetitive DNA sequences (Suzuki et al. 2007). It is possible that intragenic methylation serves a function that is conserved between invertebrates and vertebrates.

C. elegans has both similar and different epigenetic characteristics as compared to related nematode species. The free-living nematode *Pristionchus pacificus*, an established model organism in evolutionary developmental biology, contains both *dnmt2* and *mbd2*. However, no evidence exists for DNA methylation in *P. pacificus* (Gutierrez and Sommer 2004), indicating that the presence of some orthologous DNA methylation enzymes is not sufficient to predict the presence of DNA methylation. In addition, *mbd2* is present in all *P. pacificus*, *C. briggsae*, and *C. elegans*, but differences in gene sequence and structure, as well as gene function, exist (Gutierrez and Sommer 2004). *C. elegans* lacks *dnmt2*, although the loss of this enzyme was likely relatively recent in the lineage that gave rise to *Caenorhabditis*. The *C. elegans* gene *CE24669* is a potential homolog of *dnmt1*, although this protein’s function is unknown and it may have evolved a new function in the absence of DNA methylation (Gao et al. 2014). The parasitic nematode *T. spiralis* contains *dnmt1*, *dnmt2*, and *dnmt3*; notably, in a comparison of 11 nematode species, *T. spiralis* was the only one with *de novo* *dnmt3* methylation machinery, although its *dnmt3* enzyme is not closely related to mammalian *dnmt3* orthologs (Gao et al. 2014). DNA methylation is present but varies across life stages in *T. spiralis*. *T. spiralis* adult and mature muscle larval genomes have low levels of DNA methylation (1.5% cytosines are methylated) but methylation was undetectable in the newborn larval genome (Gao et al. 2014). This is in contrast to the presence of methylation in embryonic development but not adulthood in *D. melanogaster* (Capuano et al.

2014). DNA methylation may be involved in regulation of the life cycle in *T. spiralis*. Interestingly, in *T. spiralis*, transposable elements and upstream regions are more heavily methylated than other regions, a feature that was mostly thought to be absent from invertebrates and unique to vertebrate genomes.(Gao et al. 2014).

N⁶-methyl adenine: DNA N⁶-adenine methylation (N⁶-methyl adenine; 6mA) was long thought to be unique to prokaryotes, but the prevalence and significance of this mark in eukaryotes has recently been explored (Luo et al. 2015; Sun et al. 2015). 6mA has been reported in *Drosophila melanogaster* (0.001–0.07% of adenines are N⁶ methylated) and *C. elegans* (0.010.4% of adenines are N⁶ methylated) (Greer et al. 2015; Luo et al. 2015; Sun et al. 2015). Like 5-methyl cytosine, 6mA localization patterns are species-specific, possibly reflecting different functions. 6mA is found throughout the *C. elegans* genome, but is enriched at transposable elements in *D. melanogaster* (Luo et al. 2015; Sun et al. 2015). Sequence motifs in *C. elegans* (including AGAA and GAGG, representing 10% of the total methylated adenines) are different from those identified in unicellular eukaryotes and bacteria (Greer et al. 2015). Sites with high abundance of 6mA in worms are associated with GAGG and low-abundance sites with AGAA (Greer et al. 2015). Other factors beyond sequence motifs likely determine methylation status. Eukaryotic 6mA depends less strongly on sequence motif recognition than does the prokaryotic mark (Greer et al. 2015). It is unclear what role, if any, 6mA has in transcriptional control in *C. elegans*. 6mA may mark active genes in worms; 6mA levels were elevated in mutant worms with increased levels of activating histone mark H3K4me2 (Greer et al. 2015).

Consistent with the presence of 6mA, *C. elegans* encodes a potential 6mA methyltransferase *damt-1*, a member of the protein family containing an MT-A70 domain, the SAM-binding subunit that catalyzes mRNA 6mA in humans (Greer et al. 2015). This family includes yeast and mammalian mRNA methyltransferases, including methyltransferase-like protein 3 (*METTL3*) and *METTL14* in humans. Conversely, the gene *nmd-1* has been reported as a 6mA demethylase in *C. elegans* (Greer et al. 2015). In worms, deletion of the H3K4me2 demethylase, *spr-5*, leads to a progressive transgenerational loss of fertility that coincides with H3K4me2 accumulation and loss of repressive H3K9me3 (Greer et al. 2014). In *spr-5* mutants, 6mA also increases across generations and deletion of *nmd-1* accelerates the progressive fertility defect, while deletion of *damt-1* suppresses it (Greer et al. 2015). Deletion of *damt-1* also suppresses H3K4me2 accumulation, suggesting co-regulation of 6mA and H3K4me2 (Greer et al. 2015). Therefore, 6mA may function as an epigenetic mark that carries heritable information in eukaryotes (Luo et al. 2015).

b. Post-translational histone modifications and chromatin structure—The DNA sequences of core histone proteins is highly conserved, as are the sequences of enzymes that can remodel chromatin and modify histone proteins, including histone methyltransferases, acetyltransferases, demethylases and deacetylases (Corsi et al. 2015). Similar to mammals, lysine 27 (K27) and lysine 14 (K14) are among the most extensively modified residues on histone proteins (Vandamme et al. 2015). Further, data from the modENCODE project strongly support similar distribution and regulation of these epigenetic marks (Ho et al. 2014; Brown and Celniker 2015).

Patterns of histone modifications at promoters are similar and deeply conserved between worms and humans and are slightly more divergent at enhancer elements (Ho et al. 2014). Enhancers may respond to higher-order interactions between marks at a nucleosome, as human cell lines show significant variation in chromatin state at enhancers (Brown and Celniker 2015). Chromatin marks near promoters are predictive of steady-state levels of mRNA, and most marks have qualitatively similar average profiles around promoters. Quantitative relationships between chromatin state and transcript abundance are conserved between worms, and humans (Brown and Celniker 2015). Despite these commonalities, there are notable cross-species differences. For example, H4K20me1 is enriched in both active and silent genes in humans, but only in active genes in *C. elegans* (Ho et al. 2014).

Both the main effects of individual marks and combinatorial signaling can inform genome organization. For example, euchromatic regions contain H3K4me3 at the promoters and H3K36me3 in the gene body of active genes (Gerstein et al. 2010; Liu et al. 2011) while heterochromatic regions, particularly those containing repetitive elements, are enriched in methylated H3K9 (Gerstein et al. 2010; Liu et al. 2011). H3K27me3 associates with decreased transcription (Gerstein et al. 2010; Liu et al. 2011). Early-firing origins of replication (ORs) were associated with activating chromatin marks [H3K4me, H3K18ac, H3K27ac] and late-firing ORs with repressive marks [H3K9me2, H3K9me3] (Brown and Celniker 2015). However, combinations of marks, not single marks, predicted replication timing (Brown and Celniker 2015).

In *C. elegans*, heterochromatin domains are maintained via association with the nuclear lamina (Ikegami et al. 2010). The connection between laminal proximity and gene silencing is a general property of metazoans (Bank and Gruenbaum 2011). Genes in euchromatin islands within silenced domains are often highly expressed (Ikegami et al. 2010; Bank and Gruenbaum 2011). *Trans*-splicing, or the presence of alternatively spliced exons on the opposite strand from the constitutive exons, is very common in worms (Allen et al. 2011) and may be more frequent in vertebrates than previously thought (Gingeras 2009).

Studies of over 1,400 modENCODE datasets have revealed conservation of many chromatin components and mechanisms (Ho et al. 2014). Comparison of combinatorial patterns of histone modifications, nuclear lamina-associated domains, organization of large-scale topological domains, chromatin environment at promoters and enhancers, nucleosome positioning, and DNA replication patterns reveals many conserved features of chromatin organization between *C. elegans* and humans (Ho et al. 2014). Important differences include different genome sizes, chromatin architecture, and gene organization (Ho et al. 2014). A specific example is the location of heterochromatin, which tends to flank single centromeres in humans and is dispersed in heterochromatin regions along distal chromosome arms, with several centromeres spanning each chromosome (Ho et al. 2014). The composition and locations of repressive chromatin in general tend to differ between worms and humans (Ho et al. 2014).

Ho *et al* reports three distinct types of repressed chromatin that are present in flies, worms, and humans (Ho et al. 2014). The first corresponds to developmentally regulated Polycomb-silenced domains that contain H3K27me3, with little or no H3K9me3 (Ho et al. 2014). The

second corresponds to constitutive heterochromatin near the centromere in human and fly, which is absent in worms (Ho et al. 2014). The third contains both H3K9me3 and H3K27me3 and occurs primarily in worms; these repressive marks commonly co-occur in worms near heterochromatic silenced transgenes (Ho et al. 2014). In contrast to humans and flies, in which H3K9me3 is depleted at TSSs and enriched in both silent and expressed heterochromatic gene bodies, expressed genes in worm heterochromatin are depleted for H3K9me3 as compared to silent genes. In humans and flies, H3K27me3 is associated with silent genes in euchromatin, but not heterochromatin; in worms, a species-specific association between H3K27me3 and H3K9me3 leads to high levels of H3K27me3 on silent genes in heterochromatin, but moderate levels in euchromatic silent genes (Ho et al. 2014). As in mammals, germline and somatic reprogramming of these marks occurs in *C. elegans* (Kerr et al. 2014; Samson et al. 2014), but have been less robustly described in the literature, as compared to rodents and humans.

In addition, particularly in light of the robust literature on germline dynamics in worms, histone modifications are feasible mediators of epigenetic inheritance (see note on *spr-5* in 6mA paragraph). Nucleosome turnover is slower than spreading or copying of histone modifications (Dodd et al. 2007), and individual and divalent modifications (Chen et al. 2012) are sustained despite nucleosome replacement. Rapid turnover of individual histones (Deal et al. 2010) is not a barrier to transmission of epigenetic data across cell divisions, either (Brown and Celniker 2015).

c. Non-coding RNA

Small RNA: The first evidence for microRNA (miRNA) was reported in *C. elegans* (Morris and Mattick 2014); the role of miRNA was recently reviewed in (Morris and Mattick 2014) and (Hoogstrate et al. 2014), and we will highlight only a few key points here. Small regulatory RNA *lin-4* and *let-7* regulate the timing of *C. elegans* development (Morris and Mattick 2014). Although *let-7* is highly conserved from nematodes to humans, and many miRNAs are conserved, many are tissue and lineage-restricted (Morris and Mattick 2014). RNAi, which was discovered in 1998 in plants and worms, uses small interfering RNA (siRNA) processed from larger double-stranded RNA (Morris and Mattick 2014). These siRNA are similarly sized to miRNA, both interact with Dicer and RISC, and both generally function to repress translation via direct binding and targeting of mRNA for degradation (Morris and Mattick 2014). The current view is that siRNA act primarily through perfect base pairing and cleavage of complementary RNA, recommending them as experimental tools, while miRNAs act through incomplete homology at the translational level (Morris and Mattick 2014).

Structure and function of short RNAs are among the most highly conserved components of metazoan genomes (Gerstein et al. 2014), although biogenesis differs significantly across species. Worms show diverse patterns of alternative polyadenylation, which changes the length of 3' UTRs and alters miRNA-binding capacity, through development (Mangone et al. 2010). Additionally, in worms, 3' UTRs systematically shorten with age (Mangone et al. 2010). Long 3' UTRs with large numbers of binding sites for miRNA that regulate translation are common in mammals and *D. melanogaster* but have not been reported in *C.*

elegans (Miura et al. 2013). Small RNA biogenesis differs significantly among mammals, worms, and flies. Some miRNAs are processed from UTRs and mRNA coding sequence; others are derived from long primary miRNAs (pri-miRNAs) that are then processed to precursor miRNAs (pre-miRNA) (Brown and Celniker 2015). Well-conserved pre-miRNAs have been detected in nematodes and humans, but have different structures: they are shorter in worms (~0.4 kb) than in humans (>20 kb) (Brown and Celniker 2015). Mirtrons, or miRNAs spliced and processed from introns of mRNAs, have been identified in humans and nematodes, although most are lineage-specific and poorly conserved (Ruby et al. 2006; Ladewig et al. 2012). Mirtron-encoded miRNAs may have important roles in mammalian post-mitotic neurons. Additionally, in worms, short transcripts of *piwi*-interacting RNAs (piRNAs) initiate from >15,000 distinct promoters (Ruby et al. 2006; Billi et al. 2013; Hoogstrate et al. 2014). In mammals and flies, piRNAs are processed from transposable elements, long non-coding RNA (lncRNA) and 3' UTRs and introns of protein-coding transcripts. piRNA are particularly implicated in transgenerational epigenetic inheritance phenotypes in *C. elegans* (Hoogstrate et al. 2014).

Long non-coding RNA: Long-RNA with no open reading frames longer than 100 amino acids are defined as long non-coding RNA (lncRNA). Complete lncRNA maps in invertebrates enable description of diverse functions, including differential expression during differentiation and development between cell types and systems (Morris and Mattick 2014). Spatiotemporal expression patterns indicate a role in early cell fate choices (Morris and Mattick 2014). Complementary lncRNAs can base pair with mRNA, miRNA, and other lncRNA to influence mRNA stability and regulate translation (Morris and Mattick 2014). The role of lncRNA in *C. elegans* was briefly reviewed in (Morris and Mattick 2014); we will highlight a few key points here. These RNA show a wide range of conservation, from ultraconserved transcripts to primate-specific ones (Morris and Mattick 2014). Many lncRNA are structurally conserved in the absence of primary sequence conservation (Morris and Mattick 2014). Unfortunately, no studies of lncRNA antisense to protein-coding genes were conducted in worms through modENCODE, due to lack of stranded RNA data required to identify protein-coding genes transcribed on both strands (Brown and Celniker 2015).

IV. *Caenorhabditis elegans* as an emerging model system for environmental epigenetics

C. elegans is a valuable model to study the effects of environmental exposures on the epigenome, as well as developmental plasticity (reviewed in (Laubach et al. 2018)), due to its ability to respond to a variety of environmental stressors: including osmolarity (Kishimoto et al. 2017), starvation (Rechavi et al. 2014), and temperature (Klosin et al. 2017), as well as chemical pollutants, such as heavy metals (Kishimoto et al. 2017; Rudgalvyte et al. 2017), nanoparticles (Schultz et al. 2016), and others. The extent to which *C. elegans* encounters anthropogenically produced or mobilized chemicals in its natural environment is not well studied, but ongoing study of wild nematode strains will enable characterization of chemical response and resistance outside of a controlled laboratory environment, potentially permitting the adoption of this organism in ecotoxicological studies. As mentioned above, the combination of the ease of manipulation of the organism,

the high degree of conservation of epigenetic regulatory pathways and the availability of a wide range of molecular tools strongly recommends *C. elegans* for studies of the environmental influences on the epigenome, the types of epigenetic marks altered by exogenous cues, as well as the epigenetic mechanisms implicated in the nematode's response. The fast generation time and the high degree of conservation of epigenetic regulatory pathways (except cytosine methylation, as discussed above) enable the examination of multi- and trans-generational effects of environmental exposures, an exciting and compelling research topic within environmental epigenetics. In addition, since most *C. elegans* are born hermaphrodites under typical conditions, but males and/or individuals that produce only sperm or eggs can be produced by temperature shifts, as well as through genetic manipulations, *C. elegans* can be used to isolate the effects of epigenetic changes in sperm or oocytes. Foundational work in the Strome and Kelly labs distinguished the germline epigenome from the epigenome in somatic cells (Strome 2005; Furuhashi et al. 2010), and described initial evidence for transgenerational inheritance of responses to environmental stressors in *C. elegans* (Furuhashi et al. 2010; Arico et al. 2011; Kelly 2014).

Environmental epigenetics research in *C. elegans* to date has focused on the Developmental Origins of Health and Disease paradigm, or the increased risk of adult disease due to environmental exposures in early life, using one of three exposure designs: (1) somatic developmental exposure, often during larval development; (2) multi-generational effects of developmental exposure; and (3) trans-generational effects of developmental exposure. We describe these designs and summarize existing research (Table 1) using these designs below. However, we emphasize that environmental epigenetics research is not limited to designs described; the advent of single cell epigenomics techniques and the availability of a wide range of nematode strains with varying genetic backgrounds will augment an expansion of research questions in this growing sub-field.

A **multi-generational experiment** will expose several generations either directly by repeated exposure or indirectly, by exposing a gravid adult (P0) and therefore also exposing the embryo (F1) and the germline (F2) within the embryo. In contrast, a **trans-generational experiment** will expose one generation (P0) and examine a downstream generation that was never directly or indirectly exposed to the chemical (F3 and beyond). Thus, in *C. elegans*, the study of trans-generational effects at the F3 is greatly facilitated by its short generation time, reaching the F3 within 2 weeks by contrast to other common trans-generational models such as zebrafish and mice where such studies could span 6 months to a year. The study of the implication of epigenetic pathways in environmental inheritance provides by far the richest body of work on environmental epigenetics in *C. elegans*.

For example, Rudgalvyte et al used a chronic exposure paradigm throughout larval development to determine the impact of methylmercury on the epigenetic landscape (Rudgalvyte et al. 2017). The authors observed an enrichment of the active mark H3K4me3 in Phase II metabolism genes, the lipocalin-related protein gene *lpr-5*, and the cuticular collagen gene *dpy-7*, which is involved in formation of the outer cuticle, a natural barrier to chemicals (Rudgalvyte et al. 2017). Knockdown of *lpr-5* and *dpy-7* triggered increased lethality after methylmercury exposure, supporting epigenetic control of chemical defense mechanisms in the nematode (Rudgalvyte et al. 2017).

Taki *et al.* examined the impact of nicotine exposure during larval development on microRNA (miRNA) expression (Taki et al. 2014b). The authors observed that nicotine exposure was associated with differential expression of 40 miRNAs: 37 following high exposure, and 3 following low exposure, to nicotine (Taki et al. 2014b). These miRNAs clustered into distinct functional hubs, including metabolic and neuronal pathways (Taki et al. 2014b), suggesting that miRNA regulation might mediate some of nicotine's behavioral effects (Taki et al. 2013). In a follow-up transgenerational study, the authors identified a total of 14 miRNAs with differential expression by nicotine exposure across more than one generation (Taki et al. 2014a).

Schultz et al conducted a 10-generation continuous exposure study using silver ions and silver nanoparticles (Schultz et al. 2016). Continuous exposure to silver nitrate, silver nanoparticles, and sulfidized ("aged") silver nanoparticles sensitized the worms to later exposures: F2 worms showed the greatest decrease in lifespan and greatest reproductive toxicity, effects that were attenuated but sustained until the F10. While these results clearly showed a sensitization of the nematodes' response to several toxicants over many generations, the study did not explore the role of epigenetic pathways in that sensitization. There are additional reports of stressors causing heritable phenotypes, including dietary restriction (Hibshman et al. 2016).

In one of the most striking studies of transgenerational effects, Klosin *et al.* observed loss of silencing of a heterochromatic gene array that persisted for 14 generations following five generations of exposure to high temperature (Klosin et al. 2017). Even a single generation of exposure triggered loss of silencing that lasted for seven generations (Klosin et al. 2017). The inheritance occurred through both sperm and oocytes, and was associated with decreased H3K9me3 levels (Klosin et al. 2017). Gene array silencing in unexposed animals required the H3K9 methyltransferase *set-25* (Klosin et al. 2017). These results suggest that environmental exposures may perturb the balance between demethylase and methyltransferase activity required for maintenance of epigenetic patterning.

In contrast to the heat-induced effects on a repressive histone modification, arsenite exposure induces trans-generational effects on the activating mark H3K4me2. Yu and Liao observed increased H3K4me2 levels and reduced expression of the H3K4me2 demethylase, *spr-5*, for three generations following an initial developmental arsenite exposure (Yu and Liao 2016).

Active histone marks were also targeted in the response to high hormone levels. Gamez-Del-Estal et al showed that testosterone exposure induced abnormal behavioral patterns in *C. elegans*, which persisted for four generations after exposure cessation (Gamez-Del-Estal et al. 2014). Abnormal behaviors were abolished on treatment with RNAi to androgen receptor gene orthologs or with sodium butyrate, a histone deacetylase inhibitor, suggesting that activating acetylated histone proteins were required for abnormal responses.

Histone modifications are not the only epigenetic marks implicated in transgenerational inheritance. Rechavi *et al* observed that starvation-induced developmental arrest induced expression of small RNAs that were inherited for three generations (Rechavi et al. 2014). These small RNAs target genes important for nutrient reservoir activity and vitellogenins,

yolk lipoglycoproteins that provision the egg (Rechavi et al. 2014). Small RNA inheritance across required the argonaute factors *rde-4* and *hrde-1*, which are critical components of the small RNA generation pathway (Rechavi et al. 2014).

Kishimoto *et al.* addressed overlap in transgenerational mechanisms using exposure to multiple stressors in parallel (arsenite, hyperosmosis, or starvation) (Kishimoto et al. 2017). Interestingly, exposure to any of the three stressors during developmental stages resulted in increased adulthood resistance to lethal concentrations of the oxidative stressor, hydrogen peroxide (Kishimoto et al. 2017). This resistance was inherited for three generations (Kishimoto et al. 2017). Knockdown of individual H3K4 methyltransferase components, *wdr-5.1* and *set-2*, were sufficient to reverse inheritance of the resistance phenotype (Kishimoto et al. 2017). Thus all 3 stressors appeared to use a similar mechanism that involved regulation of H3K4me3 levels.

Interestingly, a recent study was able to functionally connect regulation of H3K9 methylation levels with that of small RNAs. Worms mutant for the H3K9 methyltransferase *met-2* display a progressive mortal germline phenotype in which fertility declines over many generations (10–30 generations following homozygosity) (Lev et al. 2017). Interestingly, the argonaute factor *hdre-1* is required for this progressive sterility phenotype (Lev et al. 2017). Although RNA interference (RNAi) is heritable for a few generations in *C. elegans*, *met-2* mutants show greater persistence of RNAi effects, up to 15 generations (Lev et al. 2017). This effect correlated with the presence and maintenance of small RNAs directed against the target locus across these generations (Lev et al. 2017). Thus, a model emerges in which MET-2 controls the production of small RNAs *via* the regulation of H3K9me. These exciting findings directly link repressive histone marks and small RNAs, potentially implicating both types of epigenetic mechanisms in trans-generational inheritance. Importantly, they also highlight that “forgetting” an environmental exposure response is an active process, as shown in the heightened transgenerational RNAi effects observed in *met-2* mutants.

Future research should focus on mechanisms underlying transgenerational inheritance due to environmental exposure. Specifically, due to crosstalk among epigenetic pathways, there is a great need to systematically examine the interplay between the different modes of inheritance (histone or RNA-mediated). Further, none of the aforementioned studies connected the initial environmental exposure mechanistically to reported epigenetic alterations. Further work should dissect the mechanisms linking the environmental cue with the alteration of the pathways important for epigenetic homeostasis. Last, future research should incorporate considerations of non-epigenetic parental contributions. For example, osmotic stress can alter the amount of glycogen packaged into embryos (Frazier and Roth 2009); this could be mediated by parental epigenetic changes, or accompany or induce epigenetic changes in offspring, but this could also occur with no epigenetic involvement. More broadly, stressors could simply alter loading of many protein, lipid, and nucleic acid components without epigenetic involvement. In addition, chemical pollutants may be directly loaded into embryos in exposure experiments; for example, vitellogenin can be a vector for transport of contaminants to offspring in fish (Monteverdi and Di Giulio 2000). Thus, such loading should be ruled out or considered in interpretation of data from multi- or transgenerational epigenetics experiments.

Limitations of *C. elegans* for environmental epigenetic studies

With regards to the transgenerational studies mentioned above, the relevance of the model to mammalian systems, including rodent models and human populations, has not been established yet as it is difficult to replicate the studies that can easily be performed over many generations in the nematode. In addition, some transgenerational mechanisms, including piRNA-mediated inheritance, have been less well explored in mammalian systems, and, on characterization, may be different or wholly absent from those systems. While this is not a limitation of the model *per se*, it does highlight the need for a concerted, multi-model organism approach in order to better understand the similarities, and differences, between each model. Outside of the context of transgenerational studies, the caveats to the use of the nematode for environmental epigenetics are common to all exposure approaches in the nematode: namely the difficulty in extrapolating dose-response relationships and pharmacokinetics/pharmacodynamics for application in a mammalian context, as outlined under limitations for molecular toxicology experiments. Of particular note here, it is unclear how different routes of exposure may lead to different epigenetic responses in the nematode.

V. Conclusion

The field of environmental epigenetics has expanded very rapidly since the early 2000s. The initial seminal publications in the *viable yellow Agouti* mouse model, sometimes termed an “epigenetic biosensor”, triggered broad scientific inquiry into the roles of nutritional, social and chemical environments on epigenetic patterning and reprogramming (Dolinoy and Jirtle 2008). This work has been conducted largely in rodent models, which are considered the “gold standard” for human relevance in toxicology, or through observational epidemiological studies in human populations. However, although rodents are mammals, there are clear differences between rodent and human physiology; epigenetic regulatory mechanisms are highly conserved between humans and *C. elegans*, supporting future development of *C. elegans* as a model in environmental epigenetics. Emerging themes in environmental epigenetics focus on developmental origins of health and disease, or the ability of exposures during critical developmental windows to influence risk for health outcomes in adulthood, and the role of the epigenome as an intermediate, or mediator, in the causal pathway between environmental exposures and population-level health effects. Existing evidence in these thematic areas is correlative, showing provocative associations that warrant detailed mechanistic follow-up. As the field of environmental epigenetics matures, and research begins to map mechanisms underlying observed associations, new toolkits and model systems, particularly manipulable, scalable *in vivo* systems that accurately model human transcriptional regulatory circuits, will provide an essential experimental bridge between *in vitro* biochemical experiments and mammalian model systems.

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Figure 1.
Schematic depicting strengths of *C. elegans* for environmental epigenetics.

Table 1Select studies relevant to environmental epigenetics in *C. elegans*.

Stressor	Study design	Epigenetic mark	Main findings	Reference
Methylmercury	Developmental	H3K4me3	Phase II metabolism genes, <i>lpr-5</i> , <i>dpy-7</i>	Rudgalvyte et al., 2017
Nicotine	Developmental	miRNA	37 miRNA on high exposure, 3 on low exposure	Taki et al., 2013
Arsenite, hyperosmosis, starvation	Developmental	H3K4me3	Increased adulthood resistance to hydrogen peroxide required H3K4 methyltransferase subunits <i>wdr-5.1</i> and <i>set-2</i> , overlap in mechanism of all three stressors	Kishimoto et al., 2017
Dietary restriction	Developmental	None	Altered offspring size and starvation resistance mediated by insulin-like signaling	Hibshman et al., 2016
Nicotine	Transgenerational	miRNA	14 miRNA	Taki et al., 2014
Silver ions, silver nanoparticles	Transgenerational	None	Lifespan decrease, reproductive toxicity	Schultz et al., 2016
Temperature	Transgenerational	H3K9me3	Loss of silencing of heterochromatic array required H3K9 methyltransferase <i>set-25</i>	Klosin et al., 2017
Arsenite	Transgenerational	H3K4me2	Increased H3K4me2 and reduced H3K4me2 demethylase <i>spr-5</i> expression	Yu and Liao 2016
Testosterone	Transgenerational	Post-translational histone acetylation	Abnormal behaviors abolished on RNAi to androgen receptor gene orthologs or with HDAC sodium butyrate	Gamez-Del-Estal et al., 2014
Starvation-induced developmental arrest	Transgenerational	Small RNA	Small RNA targeted nutrient reservoir activity and vitellogenins, inheritance required argonaute factors <i>rde-4</i> and <i>hrde-1</i>	Rechavi et al., 2014
Arsenite, hyperosmosis, starvation	Transgenerational	H3K4me3	Increased adulthood resistance to hydrogen peroxide required H3K4 methyltransferase subunits <i>wdr-5.1</i> and <i>set-2</i> , overlap in mechanism of all three stressors	Kishimoto et al., 2017
None	Transgenerational	H3K9me, small RNA	H3K9 mutants progressive decline in fertility, required argonaute factor	Lev et al., 2017

Stressor	Study design	Epigenetic mark	Main findings	Reference
			<i>hdre-1</i> and H3K9 methyltransferase <i>met-2</i>	

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