Integrative and Comparative Biology

Integrative and Comparative Biology, pp. 1–9
doi:10.1093/icb/icu039

Society for Integrative and Comparative Biology

SYMPOSIUM

Caenorhabditis elegans: An Old Genetic Model Can Learn New Epigenetic Tricks

Pamela A. Padilla,[1] Anastacia M. Garcia, Mary L. Ladage and Lee S. Toni

Department of Biological Sciences, University of North Texas, Denton, TX 76203, USA

From the symposium "Epigenetics: Molecular Mechanisms Through Organismal influences" presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2014 at Austin, Texas.

[1]E-mail: pamela.padilla@unt.edu

Synopsis Gamete cells pass on information to the next generation via DNA sequence and also through epigenetic mechanisms such as small RNAs, DNA methylation, or chromatin modifications. *Caenorhabditis elegans* is a genetic model system that an enormous number of talented researchers have used to understand biological phenomenon and develop molecular tools that have ultimately led to paradigm-shifting ideas in biology. Thus, this model is well poised to further investigate the molecular mechanisms involved with epigenetic modifications and transgenerational epigenetic inheritance. The strengths of this model system include a historical wealth of information regarding genetics, development, germline function, chromosome biology, and the regulation of gene expression. Using this system, one can investigate the mechanisms involved with how the germline passes on heritable epigenetic information to subsequent generations. Here, we highlight aspects about the biology of *C. elegans* that make it amenable to epigenetic studies, highlight some recent findings in the field of epigenetics, and comment on how this system would be beneficial for future biological studies involving epigenetic processes.

Caenorhabditis elegans as a genetic and epigenetic model system

The field of epigenetics involves the study of heritable changes in gene activity without a corresponding change in the DNA sequence. That is, in addition to the genetic information encoded by the DNA, gamete cells can store information in the form of epigenetic modifications, which grant plasticity to an otherwise rigid genome. At the foundation of this malleability are mechanisms that regulate gene expression and involve post-translationally modified histones or other chromatin-associated proteins, small non-coding RNAs, DNA methylation, and nuclear organization of chromatin. A growing body of evidence collected in cell culture, yeast, and animal model systems has allowed for a more complete understanding of these epigenetic regulatory mechanisms and their central role in regulating a diverse range of biological processes and phenotypes. However, the extent of heritable phenotypes attributed to epigenetic mechanisms remains to be determined and it is likely that well-characterized genetic model systems are going to play a prominent role in these discoveries.

It has been four decades since Sydney Brenner published his foundational paper on the genetic manipulation of the nematode *Caenorhabditis elegans*. With that research began a field that produced paradigm-shifting discoveries in biology (Brenner 1974; Edgar and Wood 1977). This simple 1-mm-long nematode was initially proposed as a system to study developmental genetics and indeed, key discoveries were made within this field. Examples of biological processes that were elucidated using the worm include: developmental signal-transduction pathways, how the environment works in concert with genetic pathways in regulating developmental processes such as dauer formation, the neuronal

circuitry essential to sense environmental cues, and the identification and role microRNAs have on development (Sulston and Horvitz 1977; Ellis and Horvitz 1986; Albert and Riddle 1988; Lee et al. 1993; Jorgensen and Mango 2002; Bargmann 2006; Kenyon 2010). In addition to being the first animal model system in which the genome was sequenced, a number of molecular tools, that have far reaching impact beyond *C. elegans*' biology, have been developed. Notably, RNA interference to transiently knock-down gene products and the use of green fluorescent protein to follow the expression of proteins *in vivo* were pioneered in *C. elegans* (Chalfie et al. 1994; Timmons and Fire 1998; Kamath and Ahringer 2003). The capacity to use a wide range of molecular genetic techniques, along with the multitude of biological processes that one can study, propelled the *C. elegans* model into the forefront of biological research. In the recent past, S. Brenner, J. Sulston, R. Horvitz, A. Fire, C. Mello, and M. Chalfie, using *C. elegans* to study biological processes, were awarded the Nobel Prize. Thus, given all of the molecular tools available for *C. elegans*, this model is well suited to continue a trajectory toward discovery in various fields, including epigenetics.

Recently, *C. elegans* has emerged as a genetic model system in which a mechanistic understanding of epigenetic processes is being studied, thus allowing the examination of transgenerational inheritance patterns. Relevant findings include evidence for the accumulation of age-related changes in chromatin (Wood and Helfand 2013), the contribution of nuclear organization to changes in gene expression (Haithcock et al. 2005), and heritable histone modifications and small RNAs that work through the germline (Furuhashi and Kelly 2010). Unraveling these highly conserved, and often transgenerational, epigenetic mechanisms in such an extensively characterized model system can dramatically accelerate our understanding of specific epigenetic machinery and its modifications, and their roles in determining gene expression patterns that are maintained through generations.

Undoubtedly, the environment has an effect on somatic cells, gene expression, and the overall phenotype of an organism. However, it is less understood which environmental changes induce phenotypes in subsequent generations and the mechanisms whereby that occurs. For many reasons *C. elegans* is well suited to study how the environment of the parental generation affects subsequent generations, and to identify the mechanisms that transmit gene-expression signals to the next generation (Hubbard and Greenstein 2005). First, the wealth of information about the life cycle and germline allows *C. elegans* to be amenable for epigenetic analysis (Kimble and Crittenden 2005). It has a short life cycle—needing ~3 days to develop from an embryo to a gravid adult that is either a hermaphrodite (produces ~300 offspring in a few days) or a male. The developmental progression of the germline and of gamete formation is extensively characterized; this permits investigators to expose the parental generation to experimental environments at precise time-points. The phenotypes can be followed through subsequent generations even as early as the germ-cell stage prior to fertilization. Since this model is hermaphroditic, one can study formation of both types of gametes (sperm and oocytes) in one individual prior to fertilization. The existence of males allows one to study spermatogenesis in males as well as conduct genetic crosses with hermaphrodites to analyze offspring of specific genotypes. Second, *C. elegans* has a simple anatomy with easily identifiable characteristics that can be analyzed in the context of genotype, environment, and generation. Third, it has a short lifespan (~2 weeks); thus, analysis of the lifespan of several generations can be conducted in a reasonable period of time. Finally, and most importantly, one can tightly control the environment of many generations and individuals so that one can efficiently conduct epigenetic experiments, knowing the precise environment(s) to which the animal has been exposed. Therefore, an understanding of transgenerational effects can be studied in this model, given the tools available, the knowledge of its biology, and the more recent understanding that epigenetic mechanisms involve the expression and function of small RNAs, histone modifications, and DNA methylation.

Here we note that combining the ''tried and true'' traditional and powerful genetic approaches, recent molecular techniques along with the advantages inherently part of *C. elegans*' biology allow researchers to 1) identify mechanisms that are associated with epigenetic processes and 2) describe transgenerational inheritance of phenotypes epigenetic mechanisms. Together, post-translationally modified histones, small noncoding RNAs, DNA methylation, and nuclear organization extensively regulate the activation or repression of specific genes, and ultimately regulate biological processes and allow organisms to adapt and respond to their environments. Specific examples of the role epigenetic mechanisms have in chromosomal organization, germline function,

lifespan and aging, and tolerance to stress will be discussed.

Epigenetic mechanisms and modifications of chromatin in *C. elegans*

Like all eukaryotes, *C. elegans*' genomic DNA is packaged with histone proteins to produce chromatin with a dynamic structure and function. Central to chromatin dynamics is the capacity for chromatin-modifying complexes to post-translationally modify the highly conserved N-terminal tails of core histones (H2A, H2B, H3, and H4) (Cui and Han 2007). The chromosomes of *C. elegans* are holocentric in nature, making this system somewhat unique among the genetic model systems used to study chromosome biology (Moore et al. 1999). Furthermore, chromosome biology can be easily studied in the meiotic cells of the germline and in the mitotic cells of a developing embryo, thus allowing for the analysis of chromatin modifications (Albertson and Thomson 1993; Dernburg 2001). Genome-wide analyses of histone modifications and histone variants have revealed that the structure of *C. elegans*' chromatin is remarkably similar to that of higher organisms, including mammals (Gerstein et al. 2010). In fact, the structure of all core histones is at least 80% identical in amino-acid sequence to that of human core histones (H3 and H4 proteins are 97% and 98% identical to their respective counterparts in humans), and it is hypothesized that *C. elegans* has homologs of all mammalian histone-modifying enzymes (Vanfleteren et al. 1986, 1987a, b, 1989; Gonzalez-Aguilera et al. 2013).

Chromatin modifications dramatically impact chromosome structure and function, and thus contribute to the distribution of DNA into silent heterochromatin and active euchromatin in the nucleus. The heterochromatin of *C. elegans* is enriched in what are called ''repressive'' histone modifications such as di-methylated and tri-methylated H3 at lysine 9 and 27 (H3K9me2/3 and H3K27me3), marks that are known in many organisms, to associate with transcriptionally silent DNA sequences. On the other hand, euchromatin is enriched with H3 di-methylation and tri-methylation at lysine 4 (H3K4me2, H3K4me3) (Wenzel et al. 2011). Chromatin-modifying enzymes have been shown, in some cases, to function in a complex that contain at least one histone-modifying enzyme, such as a histone methyltransferase (HMT), a histone demethylase (HDM), a histone acetyltransferase (HAT), a histone deacetylase (HDAC), or a histone kinase (Cui and Han 2007; Wenzel et al. 2011). For example, these complexes can work jointly with ATP-dependent chromatin-remodeling complexes, such as the SWI/SNF complex, and together can recognize specific histone-tail modifications. These modified histone residues can then promote remodeling and/or mobilization of nucleosomes, nucleosome exchange, incorporation of histone variants, and recruitment of small non-coding RNAs to further alter chromatin structure (Wenzel et al. 2011).

In *C. elegans* a number of specific chromatin modifiers have been identified via classical genetic approaches, RNAi screens, or by looking at multiple sequence alignments. The major chromatin-modifying enzymes predicted to be involved in modifications of chromatin include ~39 HMTs, 15 HDMs, 4 HATs, and 9 HDACs. These classes of chromatin modifiers give rise to histone modifications that are dynamic in nature, given that most chromatin modifier enzymes have been shown to have high specificity for target residues while differing in tissue-specificity and developmental requirements. Additionally, it has been shown that there exists extensive crosstalk among the different modifiers, as they are often functionally linked to reinforce the activation or repression of genes (Wenzel et al. 2011).

Chromatin-location and the inner nuclear membrane

Changes in gene expression, mediated by a variety of mechanisms including nuclear architecture, influence the phenotype of the organism (Mercer and Mattick 2013). It is known that spatial reorganization of chromatin domains relative to the nuclear periphery could impact gene expression, and in some cases an association with the inner nuclear membrane will silence gene expression. A recent study, using ChIP analysis of chromosomes of *C. elegans* embryos, showed that distal regions of autosomes associate with the transmembrane protein LEM-2. The subdomains that interacted with LEM-2 were characterized by low gene density, high levels of H3K27 trimethylation and silent genes, demonstrating that *C. elegans*' chromosomes are spatially arranged within the nucleus (Ikegami et al. 2010). A study by Towbin et al. (2012) identified mechanisms required for the sequestering of chromatin to the inner nuclear membrane. In this comprehensive work, collaborating investigators employed a combination of nested phenotype screens, transgenic strain creation, selective knockdown of gene products via RNAi, LC-MRM mass spectrometry, and immunohistochemical techniques to test their hypothesis regarding the organization of chromatin. Investigators revealed that a pair of predicted histone methyl transferases, SET-25 and MET-2, were

responsible for the stepwise methylation of Histone 3 Lysine 9 in arrays of transgenes. Additionally, the researchers determined that methylation of the H3K9 residue was the molecular signal required for the relocation and sustained anchoring of lamin-associated domains to the nuclear lamina. Towbin et al. postulated, based on compelling data and mechanistic similarities in other systems, that SET-25 may be involved in the self-reinforcing and spreading of heterochromatin domains. Future work remains to identify the mediators that recognize methylated residues and execute their relocation to the inner nuclear membrane (Towbin et al. 2012).

Chromosomal migration also occurs as a response to the stress of severe deprivation of oxygen (anoxia). Research by Padilla et al. (2002) indicates that chromatin associates with the inner nuclear periphery in cells that arrest cell division due to anoxia (Padilla and Ladage 2012). During anoxia-induced arrest of the cell cycle, a relocation of the chromosomes to the inner nuclear periphery occurs (Hajeri et al. 2005, 2010; Garcia et al. 2012). Since there is a high correlation between transcriptional reduction and association with the nuclear lamina, it is possible that this may be the result of an energy-saving mechanism that reduces gene expression. Since gene expression accounts for a considerable amount of metabolic activity, this conservation of energy may contribute to increased survival of the animal upon exit from anoxia. It remains to be seen whether stresses such as anoxia result in alterations in gene expression or in changes in chromatin that are inherited by subsequent generations.

Small RNAs, the germline, and epigenetic mechanisms

In addition to histone modifiers, small RNAs have recently emerged as profound modifiers of chromatin within the genome. *Caenorhabditis elegans* possesses two main classes of small interfering RNAs, namely 22G-RNAs and 26G-RNAs, which are anti-sense to specific coding transcripts and have been shown to regulate gene expression and chromatin modifications important in fertility and embryogenesis (Gonzalez-Aguilera et al. 2013). Additionally, Argonaute and associated small RNAs (including 22G-RNAs and 26G-RNAs) are known to function in gametogenesis. Current evidence suggests that in *C. elegans* epigenetic inheritance is mediated, at least in part, via Argonaute/small RNA pathways. Argonautes are structurally related to ribonucleases and gain sequence-specificity through these small guide RNA molecules (Conine et al. 2013). These Argonaute/small RNA complexes can direct cleavage of target mRNAs or recruit cofactors that mediate gene silencing (Ghildiyal and Zamore 2009). In *C. elegans*, the sperm can transmit both the genome and epigenetic secondary signals in the form of these Argonaute/small RNA complexes that afford a memory of gene expression in past generations (Conine et al. 2013). Moreover, it has become increasingly clear that there is dynamic and extensive crosstalk between Argonaute/small RNA pathways and chromatin modifiers that together mediate gene silencing and chromosome function (Seth et al. 2013).

The development of the germline, the process of meiosis, and the mechanisms required for proper formation of gametes to be utilized in the formation of a zygote has been extensively studied in *C. elegans* (Maine and Kimble 1990; Kimble and Crittenden 2005; Reinke 2006; Schaner and Kelly 2006; Gu et al. 2013). However, how gametes transmit epigenetic information is less understood. Recent studies have begun to elucidate how epigenetic inheritance is passed from the gamete to the zygote. It is thought that epigenetic information, in the form of RNAs and/or modified chromatin, is transmitted from the gamete to the zygote and this information can influence complex phenotypes such as stress responses and metabolism (Lippman and Martienssen 2004; Grewal and Jia 2007; Ashe et al. 2012; Buckley et al. 2012; Rando and Chang 2012; Seth et al. 2013). A recent study in *C. elegans* used traditional genetic analysis in conjunction with mRNA sequencing, and proteomic and chromatin IP analysis to demonstrate that Argonautes (ALG3/4, CSR-1) provide a paternal memory of germline gene expression (Conine et al. 2013). Argonautes direct cleavage of specific mRNAs and maintain specificity by the small RNAs (22G-RNAs) that guide the process (Ghildiyal and Zamore 2009; Buck and Blaxter 2013). Deep sequencing studies of small RNAs in wild-type and *arg-3/4* mutant alleles identified nearly 1500 genes as targeted by 26G-RNAs; a large portion of these targets had sperm-specific expression. Further analysis using mRNA-sequencing and proteomic analysis suggests that ALG-3/4 promotes the expression of targets by modulating the corresponding mRNA levels. Use of ChIP followed by Q-PCR was used to examine occupancy by RNA Pol II and concluded that negatively regulated ALG-3/4 targets are silenced at a post-transcriptional level. In this study, transcription was also analyzed in the male germline using indirect immunofluorescence to detect elongating Pol II. They found that the expression profile in spermatocytes and spermatids differed between wild-type animals and *alg-3/4* mutants, further supporting the

idea that ALG-3/4 promotes transcription of target genes in the male germline. The study then goes on to show that CSR-1 works within the ALG-3/4 pathway. By conducting various matings of heterozygous hermaphrodites and homozygous males carrying either the *alg-3/4* or *csr-1* mutant allele, and following the sterility phenotype in the heterozygotes of subsequent generations, they determined that the genes function together to provide a paternal memory of gene expression from one generation to the next. Throughout this study, to examine the phenotypes of *alg-3/4* and *csr-1* mutants they, along with wild-type controls, were subjected to various temperatures (20°C, 25°C). The researchers found that the gene-expression programs required for spermatogenesis are sensitive to temperature and that ALG-3/4 and CSR-1 are required to maintain the activation of spermatogenesis genes at elevated temperatures. In mice, testes exposed to elevated temperatures can lead to phenotypes such as impaired fertility and a decrease in the weight of progeny (Jannes et al. 1998). The study by Conine et al. (2013) provides a potential means for exploring how temperature of the parental germline could impact the phenotypes observed in offspring.

The analysis of histone modifications within the germline can help elucidate epigenetic reprogramming. In *C. elegans* one can follow chromatin modifications of germ cells throughout development. During early embryogenesis, the P4 blastomere divides to produce two primordial germ cells (called Z2 and Z3). These germ cells will arrest until later in development when division resumes and the production of gametes occurs. Analysis of chromatin in the P blastomere indicates that there are high levels of H3K4me2, yet this modification is lost when the P4 divides to produce Z2 and Z3 germ cells (Schaner et al. 2003). ChIP and western blot analysis suggest that SPR-5, which is highly expressed in the gonad and thought to be the LSD1/KDM1 ortholog, acts as a demethylase that affects H3K4me2 at target genes (Katz et al. 2009). Phenotype analysis of 28 generations of *spr-5* mutants indicates that brood size declined and sterility increased with successive generations. Furthermore, after several generations the *spr-5* mutants displayed a significant increase in H3K4me2 in the primordial germ cells indicating that across multiple generations there is inappropriate retention of modifications of chromatin within this mutant. Microarray analysis indicates that misregulation of spermatogenesis-expressed genes correlates with the sterility phenotype observed in the *spr-5* mutant. Overall, this study shows that proper removal of H3K4me2 in the primordial germ cells is important for maintenance of the germline and suggests that the germline-mortality phenotype is an epigenetic defect and not due to the accumulation of random genetic mutations.

Genetics of aging, transgenerational inheritance of aging, and epigenetics

Caenorhabditis elegans has been used extensively in studies of aging since the discovery that the animal's lifespan could be significantly extended through mutations that affect insulin-like signaling (Johnson 1990; Kenyon et al. 1993; Ogg et al. 1997). Changes in the insulin-like receptor *daf-2,* that decrease insulin-like signaling, result not only in extension of the lifespan, but also in extension of the span of healthy life (Kenyon et al. 1993; Kenyon 2010). *Caenorhabditis elegans* possesses many traits that make it amenable to studies of aging and therefore extensive studies have resulted in profound understanding of the genetic and environmental factors that influence the process of aging in this system. (Lapierre and Hansen 2012; Tissenbaum 2012). It is thought that epigenetic modifications will play a role in aging and a growing body of evidence using *C. elegans* and other models support this idea (Hamilton et al. 2005; McColl et al. 2008; Greer et al. 2011; Jin et al. 2011; Moskalev et al. 2014).

Although it has been known for some time that the insulin/IGF-1 signaling (IIS) pathway is involved in the regulation of aging in *C. elegans* and in other systems, the mechanism by which this is accomplished was not completely understood. The IIS pathway acts through a series of kinases to downregulate the function of the stress-responsive transcription factor FOXO/DAF-16. Under conditions of lowered IIS signaling either through environmental perturbation or through genetic alteration, DAF-16 nuclear localization is increased, and is associated with an increase in lifespan (Tissenbaum 2012). Recently, UTX-1 was identified as a novel regulator of the IIS pathway that epigenetically modulates the function of genes within the pathway (Jin et al. 2011; Maures et al. 2011). UTX-1 shows structural relatedness, based on amino-acid sequence, to one of two mammalian demethylases that target H3K27me3 (Jin et al. 2011). Using qPCR, RNAi, and genetic mutants, it was shown that the expression of *utx-1* increases as the animal ages, and that knockdown of *utx-1* results in a significant increase in lifespan, in a DAF-16-dependent manner. Further,

using ChIP-qPCR, it was demonstrated that UTX-1 regulates the level of H3K27me3 of the insulin/IGF-1 pathway genes *daf-2, akt-1*, and *akt-2* during aging; however, not all genes involved in aging are regulated in this way. Because H3K27me3 is a repressive epigenetic mark, its removal results in increased transcription at a given locus. In aging *C. elegans*, UTX-1 levels increase and lead to removal of the repressive H3K27me3 mark at certain genes in the IIS pathway. This removal leads to increased transcription of those genes and in turn, down-regulation of DAF-16 activity, which is associated with age-related cellular decline (Jin et al. 2011). It is interesting to note that this regulation takes place in somatic tissue, does not require a functional germline, and occurs through pathways that are highly conserved from invertebrates to mammals (Jin et al. 2011; Maures et al. 2011).

While it is known that chromatin modifiers that alter epigenetic marks are involved in regulating lifespan, transgenerational epigenetic inheritance of longevity had not been demonstrated until recently. In a study by Greer et al. (2011), researchers designed a set of experiments that addressed whether epigenetic modification of a parent generation could impact the longevity of subsequent generations. They focused on a subset of genes (*ash-2*, *wdr-5*, and *set-2*) known to regulate H3K4me3 and to extend lifespan in *C. elegans* when mutant. For each gene, the authors employed either genetic mutants or RNAi to determine whether the changes in H3K4me3 could be inherited and produce a phenotype with greater longevity (longevity phenotype). The genetic mutations were crossed out of each of the strains using wild-type males, and lifespan of the resulting progeny was documented to the fifth generation. It was shown that this particular epigenetic mark (decrease in H3K4me3) is correlated with a longevity phenotype in subsequent generations; however, there is a limit to the number of generations in which this phenotype persists. This suggests that the observed longevity phenotypes of the wild-type descendants are due to the inheritance of epigenetic changes and not a confounding factor such as extraneous mutation in the wild-type or to mutant backgrounds. It was noted that levels of H3K4me3 were not decreased in the subsequent long-lived generations; the authors hypothesize that the observed longevity is mediated through local decreases in H3K4me3 at particular loci rather than through global decreases in H3K4me3. Furthermore, the transgenerational phenotype is dependent on the expression of *rbr-2*, a demethylase that is also required for the longevity phenotype seen in the P0 generation. The authors also determined that transgenerational inheritance required the presence of a functional germline. Using microarray analysis, it was shown that a subset of genes that are differentially regulated in *wdr-5*-mutant animals continued to be differentially regulated in the wild-type descents until the fifth generation, when there was a return to normal lifespan. Of these genes, there is a subset that are known to regulate longevity and that are expressed in the germline. To date, this report is the first description of transgenerational epigenetic inheritance of longevity. Because the H3K4me3 regulatory complex under investigation is conserved from yeast to mammals, it is interesting to speculate whether transgenerational inheritance of longevity occurs in higher-order species (Greer et al. 2011). In addition to the studies described above, other recent work has shown that the FOXO/DAF-16 transcription factor acts through recruitment of the SWI/SNF chromatin remodeler. Although the process by which SWI/SNF modifies chromatin is ATP-dependent and is not mediated through epigenetic change, the recruitment of SWI/SNF is not totally dependent on DAF-16, suggesting the possibility of epigenetic regulation. It will be of interest to further investigate the relationship between epigenetic and non-epigenetic factors that influence lifespan (Riedel et al. 2013). The work reviewed here has provided an important foundation for further study, and continuing investigation will lead to deeper understanding of the role of epigenetics in aging.

Stress biology and epigenetics

Caenorhabditis elegans has been used extensively as a model for understanding the mechanistic response to changes within the environment and how animals respond to severe stress such as deprivation or overload of nutrients, deprivation of oxygen, reactive oxygen species, temperature stress, and toxins that induce a variety of cellular changes, including damage to DNA (Schulz et al. 2007; Oliveira et al. 2009; Powell-Coffman 2010; Miller et al. 2011; Ackerman and Gems 2012; Vilchez et al. 2012; Erkut et al. 2013). In many of these studies, the use of genetic analysis has elucidated stress–response mechanisms that have added significantly to our understanding of how animals respond to stress. Central to the stress–response mechanism is the ability to modulate energetic resources and produce signals that enhance survival of the stress (e.g., changes in gene expression). Post-translational modifications of a multitude of proteins are one

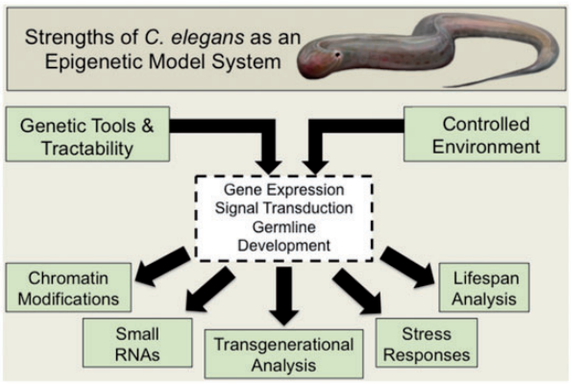

Fig. 1 *Caenorhabditis elegans* has several benefits as a model system for studying epigenetic mechanisms. The organism was historically used as a genetic model in which the genome could be manipulated and the environment tightly controlled, so detailed phenotypic analysis can be conducted. Multiple studies led to detailed understanding of many biological processes, including germline development and gene expression. These studies led to major discoveries such as microRNAs and paved the way for analyzing mechanisms involved with epigenetics.

way whereby signals can be transmitted within the cell to respond to environmental changes. For example, phosphorylation, methylation, acetylation, or O-GlcNAc modifications of proteins occur, thus affecting signal-response pathways (e.g., insulin-like signaling) or chromatin structure and function, resulting in a change in cellular homeostasis or cellular response to stress. The majority of stress–response experiments involve exposing a population of animals to a stress and then analyzing its response. We propose that *C. elegans* is an ideal model for analyzing transgenerational responses to stress to determine 1) if epigenetic mechanisms are involved with the stress response; 2) if the parental generation exposed to stress results in an effect on the subsequent generation that is not exposed to the stress; and 3) if exposure to stress for multiple generations influences the response. In considering these experiments, one must examine non-epigenetic factors to be certain that the phenotypes that are observed are not due to genetic changes. For example, DNA mutations could potentially accumulate when multiple generations are exposed to stress (e.g., temperature increases) and may be species-specific (Matsuba et al. 2013). Additionally, if gene products that are involved with DNA repair are affected (e.g., *ercc-1*, *xpf-2*, *xpg-1*, or *xpa-1*), then one may observe a decline in viability in subsequent generations due to changes within the DNA and not to epigenetic responses (Lans et al. 2013). Therefore, one has to be aware that exposure to stress could be altering phenotypes due to DNA changes and not to epigenetic changes; careful analysis would be required to distinguish among these possibilities. With the ability to conduct large genome-sequencing within *C. elegans* and have other molecular genetic tools available, *C. elegans* is in many respects an ideal model system for studying mechanisms that regulate stress responses as well as transgenerational and epigenetic responses to stress. However, a drawback to this system is that the direct relevance to more complex organisms, such as mammals, is unknown until parallel studies are conducted.

Chemicals such as methoxychlor can cause changes to gene expression that persist across multiple generations of mice (Anway et al. 2005). However, some controversy has arisen from these findings for various reasons, including ability to observe similar results (Kaiser 2014). It is less clear how molecular signals from a toxin may act in the germline to induce persistent transgenerational responses. Given that epigenetic signals are conserved across animal systems and that the detailed epigenetic mechanisms involving small RNAs and chromatin modifications are being elucidated in *C. elegans*, it may be advantageous to conduct a similar type of experiment on stress and toxins in a variety of model systems including *C. elegans*.

Conclusion

It is now several decades since *C. elegans* was initially used as a genetic model system to study developmental biology. Additionally, this model is frequently used to understand how the environment influences phenotype. Here, we reviewed several aspects of *C. elegans*' biology and some recent studies that enhanced our understanding of molecular mechanisms that are known to be involved with epigenetic processes, as well as with phenotypes that are inherited in a transgenerational manner. The availability of genetic and molecular tools, ability to tightly control the environment for many generations throughout the organisms lifespan, the ability to understand molecular mechanisms that regulate gene expression, and a detailed understanding of the germline and development makes *C. elegans* an excellent model for elucidating epigenetic mechanisms, conducting transgenerational analysis, and studying how each can influence phenotypes such as lifespan and responses to stress (Fig. 1).

Acknowledgment

We would like to thank Lacy Fenn for the artwork shown in Fig. 1.

Funding

This work was supported by a grant from the National Science Foundation (NSF CAREER 0747391) to P.A. Padilla.

References

Ackerman D, Gems D. 2012. Insulin/IGF-1 and hypoxia signaling act in concert to regulate iron homeostasis in *Caenorhabditis elegans*. PLoS Genet 8:e1002498.

Albert PS, Riddle DL. 1988. Mutants of *Caenorhabditis elegans* that form dauer-like larvae. Dev Biol 126:270–93.

Albertson DG, Thomson JN. 1993. Segregation of holocentric chromosomes at meiosis in the nematode, *Caenorhabditis elegans*. Chromosome Res 1:15–26.

Anway MD, Cupp AS, Uzumcu M, Skinner MK. 2005. Epigenetic transgenerational actions of endocrine disruptors and male fertility. Science 308:1466–9.

Ashe A, Sapetschnig A, Weick EM, Mitchell J, Bagijn MP, Cording AC, Doebley AL, Goldstein LD, Lehrbach NJ, Le Pen J, et al. 2012. piRNAs can trigger a multigenerational epigenetic memory in the germline of *C. elegans*. Cell 150:88–99.

Bargmann CI. 2006. Chemosensation in *C. elegans*. WormBook 25:1–29.

Brenner S. 1974. The genetics of *Caenorhabditis elegans*. Genetics 77:71–94.

Buck AH, Blaxter M. 2013. Functional diversification of Argonautes in nematodes: an expanding universe. Biochem Soc Trans 41:881–6.

Buckley BA, Burkhart KB, Gu SG, Spracklin G, Kershner A, Fritz H, Kimble J, Fire A, Kennedy S. 2012. A nuclear Argonaute promotes multigenerational epigenetic inheritance and germline immortality. Nature 489:447–51.

Chalfie M, Tu Y, Euskirchen G, Ward WW, Prasher DC. 1994. Green fluorescent protein as a marker for gene expression. Science 263:802–5.

Conine CC, Moresco JJ, Gu W, Shirayama M, Conte D Jr, Yates JR 3rd, Mello CC. 2013. Argonautes promote male fertility and provide a paternal memory of germline gene expression in *C. elegans*. Cell 155:1532–44.

Cui M, Han M. 2007. Roles of chromatin factors in *C. elegans* development. WormBook 3:1–16.

Dernburg AF. 2001. Here, there, and everywhere: kinetochore function on holocentric chromosomes. J Cell Biol 153:F33–8.

Edgar RS, Wood WB. 1977. The nematode *Caenorhabditis elegans*: a new organism for intensive biological study. Science 198:1285–6.

Ellis HM, Horvitz HR. 1986. Genetic control of programmed cell death in the nematode *C. elegans*. Cell 44:817–29.

Erkut C, Vasilj A, Boland S, Habermann B, Shevchenko A, Kurzchalia TV. 2013. Molecular strategies of the *Caenorhabditis elegans* dauer larva to survive extreme desiccation. PLoS One 8:e82473.

Furuhashi H, Kelly WG. 2010. The epigenetics of germ-line immortality: lessons from an elegant model system. Dev Growth Differ 52:527–32.

Garcia AM, Ladage ML, Padilla PA. 2012. Use of time lapse microscopy to visualize anoxia-induced suspended animation in *C. elegans* embryos. J Vis Exp: e4319.

Gerstein MB, Lu ZJ, Van Nostrand EL, Cheng C, Arshinoff BI, Liu T, Yip KY, Robilotto R, Rechtsteiner A, Ikegami K, et al. 2010. Integrative analysis of the *Caenorhabditis elegans* genome by the modENCODE project. Science 330:1775–87.

Ghildiyal M, Zamore PD. 2009. Small silencing RNAs: an expanding universe. Nat Rev Genet 10:94–108.

Gonzalez-Aguilera C, Palladino F, Askjaer P. 2013. *C. elegans* epigenetic regulation in development and aging. Brief Funct Genomics. [Epub ahead of print]

Greer EL, Maures TJ, Ucar D, Hauswirth AG, Mancini E, Lim JP, Benayoun BA, Shi Y, Brunet A. 2011. Transgenerational epigenetic inheritance of longevity in *Caenorhabditis elegans*. Nature 479:365–71.

Grewal SI, Jia S. 2007. Heterochromatin revisited. Nat Rev Genet 8:35–46.

Gu S, Goszczynski B, McGhee JD, Fire AZ. 2013. Unusual DNA packaging characteristics in endoreduplicated *Caenorhabditis elegans* oocytes defined by in vivo accessibility to an endogenous nuclease activity. Epigenetics Chromatin 6:37.

Haithcock E, Dayani Y, Neufeld E, Zahand AJ, Feinstein N, Mattout A, Gruenbaum Y, Liu J. 2005. Age-related changes of nuclear architecture in *Caenorhabditis elegans*. Proc Natl Acad Sci U S A 102:16690–5.

Hajeri VA, Little BA, Ladage ML, Padilla PA. 2010. NPP-16/Nup50 function and CDK-1 inactivation are associated with anoxia-induced prophase arrest in *Caenorhabditis elegans*. Mol Biol Cell 21:712–24.

Hajeri VA, Trejo J, Padilla PA. 2005. Characterization of sub-nuclear changes in *Caenorhabditis elegans* embryos exposed to brief, intermediate and long-term anoxia to analyze anoxia-induced cell cycle arrest. BMC Cell Biol 6:47.

Hamilton B, Dong Y, Shindo M, Liu W, Odell I, Ruvkun G, Lee SS. 2005. A systematic RNAi screen for longevity genes in *C. elegans*. Genes Dev 19:1544–55.

Hubbard EJ, Greenstein D. 2005. Introduction to the germ line. WormBook:1–4.

Ikegami K, Egelhofer TA, Strome S, Lieb JD. 2010. *Caenorhabditis elegans* chromosome arms are anchored to the nuclear membrane via discontinuous association with LEM-2. Genome Biol 11:R120.

Jannes P, Spiessens C, Van der Auwera I, D'Hooghe T, Verhoeven G, et al. 1998. Male subfertility induced by acute scrotal heating affects embryo quality in normal female mice. Hum Reprod 13:372–75.

Jin C, Li J, Green CD, Yu X, Tang X, Han D, Xian B, Wang D, Huang X, Cao X, et al. 2011. Histone demethylase UTX-1 regulates *C. elegans* life span by targeting the insulin/IGF-1 signaling pathway. Cell Metab 14:161–72.

Johnson TE. 1990. Increased life-span of age-1 mutants in *Caenorhabditis elegans* and lower Gompertz rate of aging. Science 249:908–12.

Jorgensen EM, Mango SE. 2002. The art and design of genetic screens: *Caenorhabditis elegans*. Nat Rev Genet 3:356–69.

Kaiser J. 2014. The epigenetics heretic. Science 343:361–3.

Kamath RS, Ahringer J. 2003. Genome-wide RNAi screening in *Caenorhabditis elegans*. Methods 30:313–21.

Katz DJ, Edwards TM, Reinke V, Kelly WG. 2009. A *C. elegans* LSD1 demethylase contributes to germline immortality by reprogramming epigenetic memory. Cell 137:308–20.

Kenyon C, Chang J, Gensch E, Rudner A, Tabtiang R. 1993. A *C. elegans* mutant that lives twice as long as wild type. Nature 366:461–4.

Kenyon CJ. 2010. The genetics of ageing. Nature 464:504–12.

Kimble J, Crittenden SL. 2005. Germline proliferation and its control. WormBook:1–14.

Lans H, Lindvall JM, Thijssen K, Karambelas AE, Cupac D, Fensgård O, Jansen G, Hoeijmakers JH, Nilsen H, Vermeulen W. 2013. DNA damage leads to progressive replicative decline but extends the life span of long-lived mutant animals. Cell Death Differ 20:1709–18.

Lapierre LR, Hansen M. 2012. Lessons from *C. elegans*: signaling pathways for longevity. Trends Endocrinol Metab 23:637–44.

Lee RC, Feinbaum RL, Ambros V. 1993. The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 75:843–54.

Lippman Z, Martienssen R. 2004. The role of RNA interference in heterochromatic silencing. Nature 431:364–70.

Maine EM, Kimble J. 1990. Genetic control of cell communication in *C. elegans* development. Bioessays 12:265–71.

Matsuba C, Ostrow DG, Salomon MP, Tolani A, Baer CF. 2013. Temperature, stress and spontaneous mutation in *Caenorhabditis briggsae* and *Caenorhabditis elegans*. Biol Lett 9:20120334.

Maures TJ, Greer EL, Hauswirth AG, Brunet A. 2011. The H3K27 demethylase UTX-1 regulates *C. elegans* lifespan in a germline-independent, insulin-dependent manner. Aging Cell 10:980–90.

McColl G, Killilea DW, Hubbard AE, Vantipalli MC, Melov S, Lithgow GJ. 2008. Pharmacogenetic analysis of lithium-induced delayed aging in *Caenorhabditis elegans*. J Biol Chem 283:350–7.

Mercer TR, Mattick JS. 2013. Understanding the regulatory and transcriptional complexity of the genome through structure. Genome Res 23:1081–8.

Miller DL, Budde MW, Roth MB. 2011. HIF-1 and SKN-1 coordinate the transcriptional response to hydrogen sulfide in *Caenorhabditis elegans*. PLoS One 6:e25476.

Moore LL, Morrison M, Roth MB. 1999. HCP-1, a protein involved in chromosome segregation, is localized to the centromere of mitotic chromosomes in *Caenorhabditis elegans*. J Cell Biol 147:471–80.

Moskalev AA, Aliper AM, Smit-McBride Z, Buzdin A, Zhavoronkov A. 2014. Genetics and epigenetics of aging and longevity. Cell Cycle 13:1063–1077.

Ogg S, Paradis S, Gottlieb S, Patterson GI, Lee L, Tissenbaum HA, Ruvkun G. 1997. The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in *C. elegans*. Nature 389:994–9.

Oliveira RP, Porter Abate J, Dilks K, Landis J, Ashraf J, Murphy CT, Blackwell TK. 2009. Condition-adapted stress and longevity gene regulation by *Caenorhabditis elegans* SKN-1/Nrf. Aging Cell 8:524–41.

Padilla PA, Ladage ML. 2012. Suspended animation, diapause and quiescence: arresting the cell cycle in *C. elegans*. Cell Cycle 11:1672–9.

Padilla PA, Nystul TG, Zager RA, Johnson AC, Roth MB. 2002. Dephosphorylation of cell cycle-regulated proteins correlates with anoxia-induced suspended animation in *Caenorhabditis elegans*. Mol Biol Cell 13:1473–83.

Powell-Coffman JA. 2010. Hypoxia signaling and resistance in *C. elegans*. Trends Endocrinol Metab 21:435–40.

Rando TA, Chang HY. 2012. Aging, rejuvenation, and epigenetic reprogramming: resetting the aging clock. Cell 148:46–57.

Reinke V. 2006. Germline genomics. WormBook:1–10.

Riedel CG, Dowen RH, Lourenco GF, Kirienko NV, Heimbucher T, West JA, Bowman SK, Kingston RE, Dillin A, Asara JM, et al. 2013. DAF-16 employs the chromatin remodeller SWI/SNF to promote stress resistance and longevity. Nat Cell Biol 15:491–501.

Schaner CE, Deshpande G, Schedl PD, Kelly WG. 2003. A conserved chromatin architecture marks and maintains the restricted germ cell lineage in worms and flies. Dev Cell 5:747–57.

Schaner CE, Kelly WG. 2006. Germline chromatin. WormBook:1–14.

Schulz TJ, Zarse K, Voigt A, Urban N, Birringer M, Ristow M. 2007. Glucose restriction extends *Caenorhabditis elegans* life span by inducing mitochondrial respiration and increasing oxidative stress. Cell Metab 6:280–93.

Seth M, Shirayama M, Gu W, Ishidate T, Conte D Jr, Mello CC. 2013. The *C. elegans* CSR-1 Argonaute pathway counteracts epigenetic silencing to promote germline gene expression. Dev Cell 27:656–63.

Sulston JE, Horvitz HR. 1977. Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans*. Dev Biol 56:110–56.

Timmons L, Fire A. 1998. Specific interference by ingested dsRNA. Nature 395:854.

Tissenbaum HA. 2012. Genetics, life span, health span, and the aging process in *Caenorhabditis elegans*. J Gerontol A Biol Sci Med Sci 67:503–10.

Towbin BD, Gonzalez-Aguilera C, Sack R, Gaidatzis D, Kalck V, Meister P, Askjaer P, Gasser SM. 2012. Stepwise methylation of histone H3K9 positions heterochromatin at the nuclear periphery. Cell 150:934–47.

Vanfleteren JR, Van Bun SM, Delcambe LL, Van Beeumen JJ. 1986. Multiple forms of histone H2B from the nematode *Caenorhabditis elegans*. Biochem J 235:769–73.

Vanfleteren JR, Van Bun SM, Van Beeumen JJ. 1987a. The primary structure of histone H3 from the nematode *Caenorhabditis elegans*. FEBS Lett 211:59–63.

Vanfleteren JR, Van Bun SM, Van Beeumen JJ. 1987b. The primary structure of histone H4 from the nematode *Caenorhabditis elegans*. Comp Biochem Physiol B 87:847–9.

Vanfleteren JR, Van Bun SM, Van Beeumen JJ. 1989. The histones of *Caenorhabditis elegans*: no evidence of stage-specific isoforms. An overview. FEBS Lett 257:233–7.

Vilchez D, Morantte I, Liu Z, Douglas PM, Merkwirth C, Rodrigues AP, Manning G, Dillin A. 2012. RPN-6 determines *C. elegans* longevity under proteotoxic stress conditions. Nature 489:263–8.

Wenzel D, Palladino F, Jedrusik-Bode M. 2011. Epigenetics in *C. elegans*: facts and challenges. Genesis 49:647–61.

Wood JG, Helfand SL. 2013. Chromatin structure and transposable elements in organismal aging. Front Genet 4:274.