

C. elegans epigenetic regulation in development and aging

Cristina González-Aguilera, Francesca Palladino and Peter Askjaer

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Abstract

The precise developmental map of the *Caenorhabditis elegans* cell lineage, as well as a complete genome sequence and feasibility of genetic manipulation make this nematode species highly attractive to study the role of epigenetics during development. Genetic dissection of phenotypical traits, such as formation of egg-laying organs or starvation-resistant dauer larvae, has illustrated how chromatin modifiers may regulate specific cell-fate decisions and behavioral programs. Moreover, the transparent body of *C. elegans* facilitates non-invasive microscopy to study tissue-specific accumulation of heterochromatin at the nuclear periphery. We also review here recent findings on how small RNA molecules contribute to epigenetic control of gene expression that can be propagated for several generations and eventually determine longevity.

Keywords: *Caenorhabditis elegans*; chromatin organization; longevity; organogenesis; small RNA; transcriptional silencing

INTRODUCTION

Several characteristics of *Caenorhabditis elegans* make this small free-living nematode an ideal model organism to understand how epigenetic mechanisms impinge on developmental processes. Note that we here define epigenetics broadly to include the activity of any chromatin-modifying proteins. After Sidney Brenner established the first protocols to efficiently screen for abnormal behavioral phenotypes [1], a wide variety of mutations have been mapped genetically. Several of the corresponding genes have subsequently been shown to encode members of chromatin-modifying complexes, such as NuRD acting during vulva formation [2] and Polycomb affecting germline survival [3], thus providing molecular insight as to how epigenetic changes may control development. The *C. elegans* compendium of chromatin-modifying enzymes was recently reviewed [4, 5] and we emphasize here novel discoveries.

Despite its simple body morphology and absence of several tissues types found in more sophisticated animals, a high proportion of human protein coding

genes are conserved in *C. elegans* [6]. In particular, most major signaling pathways are present and vulval development has become a paradigm of organogenesis controlled by Ras/RTK and Wnt signaling; several components of these pathways were indeed first described in *C. elegans* [7]. During larval development, three of six vulval precursor cells (VPCs) are specified to become vulva cells. However, if Ras/RTK signaling is hyperactivated, or, as described below, if chromatin-modifying complexes are deregulated, additional precursor cells may be induced, leading to the formation of extra pseudovulvae. Another developmental event for which *C. elegans* has attracted significant attention is the decision in early development to either continue the normal larval program or to enter a resistant diapause stage known as dauer [8]. Dauer larvae are able to survive starvation and other unfavorable conditions for prolonged periods of time and show a several-fold increase in absolute lifespan. As we discuss, this is also an interesting example of how developmental plasticity correlates with changes in chromatin states.

Corresponding author. Peter Askjaer, Centro Andaluz de Biología del Desarrollo (CABD), CSIC/JA/Universidad Pablo de Olavide, Ctra de Utrera Km1, 41013 Seville, Spain. Tel.: +34-954-348-396; Fax: +34-954-349-376; E-mail: pask@upo.es

Cristina González-Aguilera is a postdoctoral fellow at the Biotech Research & Innovation Centre (BRIC) in Copenhagen, Denmark. She focuses on chromatin maintenance and epigenetic mark restoration during cell division.

Francesca Palladino is a group leader at the Ecole Normale Supérieure de Lyon, France. Her main research interest is how chromatin regulation influences germline development and specific stress responses in the soma.

Peter Askjaer is a group leader at the Andalusian Center for Developmental Biology (CABD) in Seville, Spain. He studies nuclear envelope proteins and their roles in nuclear organization, gene expression and development.

Development from zygote to fertile adult takes typically 3 days under standard laboratory conditions, followed by 15–20 days of adulthood in which the hermaphroditic nematode produces 200–300 offspring. From these numbers, one can infer that *C. elegans* provides both a rich resource of rapidly dividing germ cells and embryos as well as populations of animals whose lifespan can be studied in a short period of time. Several landmark discoveries were indeed made under these opposite extremes of *C. elegans* life history, including the identification of PAR polarity genes in early embryos and longevity-inducing mutants of the insulin/IGF signaling pathway in adults [9, 10]. Interestingly, proper regulation of histone modifications is important both to maintain germ cells in their native state and to specify normal lifespan: depletion of chromatin-modifying factors was recently linked to premature differentiation of germ cells [11] and to longevity extension [12].

The possibility of experimentally inhibiting gene expression by RNA interference (RNAi) boosted *C. elegans* popularity ~15 years ago. For several reasons, *C. elegans* is still an attractive choice to perform RNAi experiments. Efficiency is typically very high due to an endogenous amplification step and experiments can readily be scaled up to genome-wide analysis in a time and cost-effective manner [13]. Research into the mechanism of RNAi led to the discovery of a multitude of naturally occurring small RNA-mediated processes, including transcriptional silencing, messenger RNA degradation and translational regulation. Remarkably, several of these effects can be inherited for generations, even in the absence of the initial trigger, and we describe how this may relate to epigenetic modifications.

The organization of the *C. elegans* genome into heterochromatic and euchromatic domains is similar to that in other eukaryotes and we open the chapter with a description of how *C. elegans* was used to identify a mechanism responsible for the enrichment of heterochromatin at the nuclear periphery.

HETEROCHROMATIN AND THE NUCLEAR PERIPHERY

The traditional description of the nucleus as being divided into a peripheral region containing silent heterochromatin and an interior region containing actively transcribed euchromatin has gained complexity in the last years. Advances in fluorescence

and live microscopy and the development of new genome-wide techniques have started revealing the complexity of the 3D-spatial distribution of chromatin inside the nucleus. This includes loops in the DNA molecule and interaction with nuclear compartments such as the nuclear envelope (NE) and the nucleolus (reviewed in [14]). However a main question remains unanswered in the field: is the spatial distribution of chromatin the cause or the consequence of changes in gene expression? Furthermore, it remains unknown how chromatin organization affects developmental processes. *C. elegans* has become an excellent model to address these open questions, not only because of the conservation of chromatin structure with others eukaryotes but also because of the ease of genetic manipulation and body transparency. Here we will mainly focus on recent discoveries of the importance of histone modifications in the anchoring of chromatin to the nuclear periphery and gene silencing.

The 97 megabases of the *C. elegans* genome are distributed in six chromosomes; five pairs of autosomes and the X chromosome found in two copies in hermaphrodites or one copy in males [15]. Genome-wide analyses of more than 19 histone marks and histone variants have revealed that the structure of *C. elegans* chromatin is similar to mammals and flies. Epigenetic marks associated with active chromatin such as trimethylation of lysine 4 or lysine 36 on histone 3 (H3K4me3 and H3K36me3) are enriched in active promoters and gene bodies, respectively, while H3K27me3 is associated with repressed genes [16]. H3K9 methylation, a heterochromatic mark typically associated with pericentromeric regions in monocentric organisms, presents a particular distribution in *C. elegans*, being associated with chromosome arms in large domains expanding up to 4 Mb and absent from chromosome centers [16, 17]. A possible explanation for this different distribution could be the holocentric nature of *C. elegans* chromosomes that lack a defined heterochromatic centromeric region; instead this function is distributed along the length of the chromosomes [18]. Moreover, H3K9 methylation is enriched on repetitive DNA and regions with a high recombination rate, as in flies and mammals [16, 19].

Consistently, both genome-wide analysis based on ChIP experiments of the inner nuclear membrane protein LEM-2/MAN1, and DamID experiments of the only component of the nuclear lamina, LMN-1,

demonstrated that, in *C. elegans* embryonic nuclei, the NE associates with autosomal arms [20, 21] (Figure 1). For the X chromosome, association with the NE was only observed at the end of the left chromosome arm, consistent with the different distribution of repressive histone marks and the reduced expression levels of genes in this region, most likely due to dosage compensation complex activity [16]. This indicates that the general association of heterochromatin with the NE observed in humans [22] is conserved in *C. elegans*.

Does anchoring to the NE affect gene expression? Genes found within NE-associated domains in nematodes, flies and mammals generally have lower expression levels than those in other chromatin domains [20, 22, 23]. In addition, within the large *C. elegans* heterochromatin domains one finds small regions of chromatin detached from the NE and showing high expression levels [20]. These experiments suggest that the anchoring to the nuclear periphery could trigger gene repression. However, only recently, through the development of a novel system to model heterochromatin in *C. elegans*, have we

started to really understand the mechanism by which chromatin can be attached to the NE and promote silencing. The system is based on genome-integrated artificial long transgene arrays that can be visualized by the Lac Operator/LacI-GFP system. Interestingly, these arrays contain the typical heterochromatin marks such as H3K9me3 and H3K27me2/3 and are sequestered at the NE and silent during embryogenesis, but move to the nuclear interior when activated during larval development. [24]. An RNAi screen that combined the use of this heterochromatin system with fluorescence microscopy, identified two H3K9 methyltransferases, MET-2, the homolog of the human SETDB1 and SET-25, similar to human EHMT2/G9a, as the main players in the anchoring and silencing of heterochromatin regions at the NE [21] (Figure 1). MET-2 deposits H3K9me1/2 on cytoplasmic free histones. The exact mechanism of how these marks are loaded onto DNA is not known, but once incorporated into chromatin, the presence of these marks leads to the anchoring of chromatin to the NE, although it does not cause gene repression. SET-25

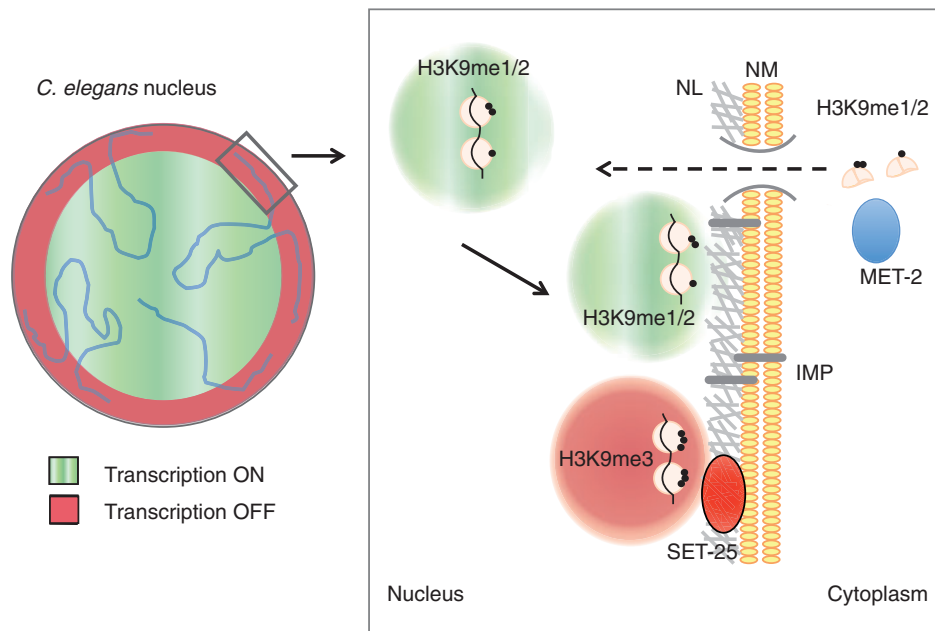


Figure 1: Silencing of heterochromatin at the NE. In *C. elegans* embryos, heterochromatin is distributed at the end of chromosome arms and is placed close to the NE whereas the transcriptionally active central part of the chromosomes is located in the nuclear interior. Two sequential steps involving H3K9 methylation are needed for the anchoring and silencing of heterochromatin at the NE. First, in the cytoplasm, the histone methyltransferase MET-2 produces H3K9me1/2, presumably as free H3–H4 dimers. These dimers, once translocated into the nucleus and incorporated into chromatin to form nucleosomes, facilitate the anchoring to the NE but do not result in transcriptional repression. The activity of SET-25, a G9a-like histone methyltransferase present at the NE, then catalyzes the H3K9me3 reaction and promotes silencing. NM, Nuclear Membrane; NL, Nuclear Lamina; IMP, Integral Membrane Protein.

located at the NE then converts H3K9me1/2 to H3K9me3 and promotes silencing [21]. Interestingly, the implication of sequential H3K9 methylation steps in the anchoring and silencing of heterochromatin appears to be conserved in others eukaryotes, since similar results have been observed in mice [25]. However, the fact that the *met-2 set-25* double mutant is viable despite having no detectable methylated H3K9, and that not all the heterochromatin is detached from the NE in these embryos, suggests that alternative mechanisms have to exist. These may include redundant activity with other histone marks and the differential activity of NE components which may compensate each other, as suggested for Lamin A/C and the Lamin B receptor protein (LBR) in rodents [26]. In addition, non-coding RNAs may also be involved, since the small interfering RNA (siRNA) pathway directs H3K9 methylation in *C. elegans* [27, 28] and plays an important role in heterochromatin formation at repetitive DNA elements surrounding centromeres in the fission yeast *Schizosaccharomyces pombe* (Reviewed in [29]; see below).

The presence of alternative mechanisms fits with the idea that chromatin organization is a dynamic process that has to respond to changes in the environment. Indeed, movements of stress-induced genes from the nuclear interior toward the nuclear pore complex upon activation have been observed both in nematodes and yeast [30, 31]. However, the most important changes in nuclear organization have been found during development. In mammals, comparison of lamina-associated domains during the differentiation of mouse embryonic stem cells to mature astrocytes revealed hundreds of regions with altered lamina binding, including individual genes but also large domains affecting many loci [32]. Interestingly, there is evidence indicating that different mechanisms may control chromatin organization during development and between tissues. One example is the unequal contribution of Lamin A/C and LBR to the anchoring of chromatin to the NE in rodents. During early embryogenesis LBR is the mayor player while Lamin A/C becomes more important later in development in a tissue-specific manner [26]. In nematodes, movement of newly expressed genes from the NE toward the nuclear interior have also been observed after tissue-specific activation [24]. Besides, NE components seem to regulate chromatin structure in a tissue-specific way. An example is provided by the inner nuclear membrane protein

EMR-1/Emerin that in *C. elegans* negatively regulates the activity of the foregut transcription factor PHA-4 in the pharynx but not in the intestine, presumably affecting chromatin compaction [33]. Moreover, genome-wide DamID analysis in adult nematodes has revealed that EMR-1 is associated preferentially with muscle and neuronal genes (C.G.-A. and P.A.; unpublished). However, histone marks related to heterochromatin and silencing are not the only ones to show developmental and tissue-specific changes. The composition and specificity of the nematode SET1/MLL-related H3K4 histone methyltransferase complex changes during tissue differentiation: while SET-2 and ASH-2 are differentially required for H3K4me2/3 in the adult germline, H3K4me2 is mostly dependent on ASH-2 in embryos [34, 35]. These examples illustrate the necessity of future tissue-specific analyses to decipher the role of the NE in chromatin organization and histone modification during development.

SYNMUV GENES AND CELL-FATE DECISIONS IN *C. ELEGANS* DEVELOPMENT

Genetic screens based on the multivulva (Muv) phenotype were instrumental in first characterizing chromatin associated factors in *C. elegans*. Vulval cell-fate specification in *C. elegans* requires both activation of an RTK/Ras/Map signaling pathway and negative regulation by a set of genes known as the synMuvs, based on the synthetic Muv phenotype displayed when a class B mutation is combined with a class A [36]. The synMuv B class defines a distinct set of genes encoding conserved transcriptional repressors and chromatin-associated factors, including the Rb transcriptional-regulatory complex, the HP1 homolog HPL-2 and components of the NuRD complex.

Mutations in many synMuv B genes also cause misexpression of germline-specific P granules in somatic cells and enhanced RNAi [37, 38]. These phenotypes may be linked, since the enhanced RNAi response in these mutants could result from the somatic misexpression of germline-specific genes, including many RNAi factors which are preferentially expressed in the *C. elegans* germline, which is also most proficient for RNAi [39].

Loss of global regulators of germline chromatin, including the MES-4 H3K36 methyltransferase,

MRG-1, the ISW-1 chromatin remodeling factor and the Polycomb Repressor Complex 2 (PRC2) components MES-2/3/6 suppressed the somatic misexpression in *synMuv* animals, suggesting a general role for *synMuvB* genes in preventing cells from adopting a chromatin structure permissive for germline-like fate [39, 40]. While ectopic expression of germline genes in the soma of *synMuv B* mutants does not affect viability under normal growth conditions (20°C), a recent study [41] showed that when grown at high temperature (26°C), a majority of *synMuv B* mutants irreversibly arrest at the L1 stage. High temperature arrest was accompanied by upregulation of many germline genes, and also suppressed by mutations in the germline chromatin factors *mes-4*, *mrg-1* and *isw-1*. Altogether, these results suggest that larval arrest is caused by somatic cells acquiring a germline-like chromatin state through an inherently temperature sensitive process.

Interestingly, links between temperature, chromatin state and gene expression are apparent in both plants, where temperature-dependent incorporation of the histone variant H2A.Z is crucial for the changes in gene expression that underlie high temperature growth phenotypes [42], and in *Drosophila*, where growth at high temperature suppresses position effect variegation [43], and affects plasticity of pigmentation [44].

SMALL RNA-MEDIATED CHROMATIN SILENCING IN *C. ELEGANS*

Over the past few years, small RNAs have emerged as the primary signal required for directing distinct chromatin modification in the genome, thereby regulating the plasticity of the epigenome. *C. elegans* has two main classes of small interfering RNAs (endo-siRNAs), named 22G-RNAs and 26G-RNAs, which start with a G and are 22 and 26 nt in length, respectively [45–47]. These siRNAs are anti-sense to coding transcripts and have been implicated in regulating fertility and embryogenesis via regulation of gene expression and chromatin modifications [48]. 26G-RNAs are primary siRNAs produced by Dicer, while the more abundant 22G-RNAs are secondary siRNAs which depend on the RNA-dependent RNA polymerases (RdRPs) EGO-1 or RRF-1 for biogenesis. Although most 22G-RNA-dependent silencing mechanisms characterized thus

far occur in the germline, a recent study suggests they may also play a role in the soma [49]. Depending on their function, 22G-RNAs associate with distinct AGO (Argonaute) proteins: NRDE-3 (in the soma) or HRDE-1 (in the germline), to target nascent RNAs to the nucleus and regulate histone H3K9 methylation [27, 50, 51], WAGO-1 in the germline, to regulate gene expression and transposon silencing [52], and CSR-1 both in germline and soma to regulate chromatin structure and chromosome segregation [53].

In the soma, NRDE-3 (Nuclear RNAi Deficient), associates with a subset of endogenously produced 22G-RNAs or with secondary siRNAs generated from the administration of double-stranded RNA (dsRNA) during experimentally induced exogenous RNAi. The NRDE-3/siRNA complex then translocate into the nucleus where it associates with nascent transcripts at genomic loci complementary to the small RNAs [51] and recruits the conserved nuclear factor NRDE-2. The *Nrde* pathway inhibits RNA Polymerase (RNAP) II during the elongation phase of transcription and directs the deposition of H3K9 trimethylation at target sites [27, 54, 55]. Endogenous siRNAs together with NRDE-3 and other nuclear RNAi pathway components were recently shown to promote odor adaptation in *C. elegans* olfactory neurons, suggesting that these small RNAs may provide an exciting link between environmental stimuli and behavioral changes [49].

Endogenous small 21U-RNAs (21 nt-long RNAs with a 5' U) that interact with the conserved Piwi protein PRG-1 and are the *C. elegans* equivalent of piRNAs have also been linked to chromatin-regulatory mechanisms [56–58]. 21U-RNAs map to two large genomic clusters and target aberrant and coding transcripts broadly, triggering production of secondary endogenous 22G-RNAs [45, 46, 59]. 22G-RNAs associate with WAGOs and form target-specific endo-siRNAs, which interact with nascent transcripts in the nucleus to induce co-transcriptional gene silencing (coTGS). By using a single-copy transgenic reporter with a long exogenous DNA sequence, Shirayama *et al.* [58] showed that silencing was dependent on nuclear and cytoplasmic WAGOs and correlated with the accumulation of 22G-RNAs targeting the foreign portion of the transgene. PRG-1 was required to initiate, but not to maintain, silencing of the reporter. These observations have led to a model whereby *C. elegans* piRNAs scan

the germline transcriptome to identify and silence foreign sequences. Additional studies showed that silencing involves establishment of repressive chromatin marks which contribute to continued gene silencing, even in subsequent generations [28, 54, 56].

MAINTENANCE OF TRANSCRIPTIONAL SILENCING AND TRANSGENERATIONAL INHERITANCE

Gu *et al.* [28] showed that following exogenous RNAi, both the small RNAs and H3K9 methylation that were initiated in the parents are transmitted to progeny. The authors also showed that H3K9me3 at the target locus persisted for two generations without any additional input of dsRNA, while the level of small RNAs rapidly dropped after the first generation, consistent with small RNAs bringing about histone modifications. At the loci targeted by exogenous RNAi, the highest levels of H3K9me3 are acquired proximal to the dsRNA trigger, and spread up to 9 kb. The mechanisms required for limiting the extent of H3K9me3 spreading however, have not been identified.

piRNAs can also trigger highly stable long-term silencing lasting at least 20 generations [56]. Once established, this long-term memory becomes independent of PRG-1 and the piRNA trigger, but remains dependent on the nuclear RNAi/chromatin pathway including a germline-specific nuclear Argonaute HRDE-1/WAGO-9, the previously identified NRDE proteins, the HP1 ortholog HPL-2, and two histone methyltransferases, SET-25 and SET-32 [27, 50, 54, 56]. This is accompanied by accumulation of H3K9me3 at the genomic locus that encoded the target RNAs. Interestingly, germ cells of WAGO-9 mutants lose their characteristic immortality and become sterile after several generations, a phenomenon accompanied by loss of H3K9me3 [50]. PRG-1 may therefore act through WAGO-9 to stably maintain proper gene expression in the germline over generations. Progressive sterility is also observed in the absence of H3K4 methylation in the germline [35], although it is not known whether deposition of this mark is also associated with small RNAs. Small RNA silencing mechanisms and chromatin structure may therefore play a wider role in the maintenance of an immortal germline,

perhaps through the silencing of genes incompatible with sustained germline function.

CSR-1 AND HOLOCENTRIC CHROMOSOMES

While loss of most individual Argonaute proteins results in minor immediate phenotypes, *csr-1* is required for normal chromosomal segregation and its absence results in aneuploidy and embryonic lethality. CSR-1 is found in a complex consisting of EGO-1, DRH-3 and EKL-1, which is responsible for the synthesis of 22G-RNAs and chromosome segregation [53]. CSR-1 is enriched at specific chromosomal loci in a small RNA and target transcript-dependent manner, suggesting that it may interact with nascent transcripts at these loci. A variety of 4200 germline-expressed protein coding genes dispersed in clusters along the length of each chromosome are targeted by the CSR-1 pathway [53], consistent with a role in the formation of holocentromeres along the lengths of *C. elegans* chromosomes. CSR-1/22G-RNA target genes are not generally downregulated, but are characterized by histone modifications that correlate with active chromatin, including H3K4me2,3 and H3K36me2,3 [60]. At present it remains unknown whether these modifications simply reflect ongoing transcription, or a direct involvement of CSR-1 in the deposition of these active marks on target genes, in contrast to the heterochromatin-associated marks directed by the RNA pathways previously discussed. It was proposed that CSR-1 might affect chromosome segregation through centromere formation [53]. In support of this, at least some members of the CSR-1 complex display a localization pattern on holocentric *C. elegans* chromosomes reminiscent of kinetochores [53, 61]. However, although kinetochore organization is aberrant in *csr-1* mutant embryos loading of kinetochore components onto mitotic chromosomes is not inhibited [53, 61]. Consistent with this latter observation, global mapping of kinetochore protein HCP-3/CENP-A binding sites indicates that CSR-1 and HCP-3 associate to separate chromatin regions [62]. A possible model for CSR-1 function in chromosome segregation thus involves association to a multitude of 22G-RNA target sites thereby affecting globally the epigenetic landscape, and, as consequence, chromosome organization and congression during mitosis.

REGULATION OF THE DAUER DEVELOPMENTAL DECISION BY CHROMATIN FACTORS AND RNAI

The *C. elegans* dauer stage is one of the most striking examples of developmental plasticity in which environmental cues influence developmental decisions [8]. *Caenorhabditis elegans* enters the dauer stage in response to harsh environmental conditions, including starvation, crowding and elevated temperatures. Dauer animals can re-enter the reproductive life cycle once conditions improve. Remarkably, recent experiments have shown that animals that have transited through the dauer stage (post-dauer) show specific phenotypes, altered gene expression and global changes in chromatin modifications compared to animals maintained under favorable conditions [63, 64]. These results suggest that post-dauer animals retain a cellular memory of their developmental history.

Using ChIP-Seq analysis, Hall *et al.* [63] went on to show that the changes in expression profiles observed in dauer and post-dauer animals are associated with global alterations in active chromatin marks, including H3K4 methylation and H4 acetylation. Overall gene expression levels, however, remained similar, and no correlation was observed between the fold change in expression and the chromatin modification profiles when comparing control and post-dauer population. Based on these observations, the authors suggested that global histone modifications may poise genes for further regulation by additional mechanisms. Despite the absence of a direct correlation between global changes in gene expression and specific chromatin marks, for a number of genes whose expression was found to change in post-dauer animals, this effect was dependent on chromatin factors, including the histone deacetylase *hda-2* and the HP1 homologs HPL-1 and HPL-2. Therefore, changes in chromatin structure may play a causal role in the establishment or maintenance of the post-dauer expression changes.

In a follow up study, deep sequencing was used to profile endogenous siRNAs from post-dauer and control adults, identifying hundreds of genes with significant changes in endo-siRNAs, a subset of which also showed changes in gene expression [64]. Comparison with previously published gene sets showed that genes exhibiting increased siRNA levels in post-dauer were more likely to be targets of the CSR-1 and EGO-1 pathways associated with 22G-RNAs, while genes exhibiting decreased siRNA levels were targets of 26G-RNAs. CSR-1

was shown to play a major role in the regulation of gene expression changes in wild-type post-dauer animals. Mutations in additional specific endo-siRNA pathway factors disrupted gene expression changes in post-dauer animals for a subset of these genes, and also affected the increased brood size phenotype of post-dauer adults. Moreover, H3K4me3 and H4ac were dramatically increased at a subset of examined loci in post-dauer animals lacking *csr-1*, suggesting that CSR-1 is a key effector in modulating chromatin state changes upon passage through the dauer stage.

Altogether, these studies suggest that changes in chromatin structure and RNAi may underlie the establishment or maintenance of a memory of developmental history. It remains to be established how these changes are related to the different temporal patterns of gene expression changes in post-dauer animals. Furthermore, the observation that in a sensitized background dauer animals lacking HPL-2 are unable to re-enter the reproductive lifecycle under favorable conditions [65], suggests that chromatin regulation may play a wider role in regulating the dauer developmental decision.

GERM CELL TOTIPOTENCY AND TRANSDIFFERENTIATION

Establishment and maintenance of germline totipotency is a universal requirement for species survival. From an epigenetic point of view, germ cells represent a ground state from which differentiation into particular cell types is accompanied by specific DNA and chromatin modifications [66]. Differentiation relies on transcription factors to activate complex gene-regulatory networks relevant for the given cell and tissue type. Many transcription factors have been identified as being required at specific steps in individual differentiation programs, but are often dependent on cellular environment and co-regulators. A recent study found that chromatin modifications are most likely to be at least partly responsible for this limitation. In an attempt to produce terminally differentiated neurons the Hobert laboratory discovered that expression of the transcription factor CHE-1 was only able to promote ectopic formation of ASE neurons in the absence of the histone chaperone LIN-53 (RBBP4/RBBP7) [11]. Remarkably, ASE neurons could be directly generated from germ cells, and, moreover, other neuronal transcription factors were able to induce different types of neurons

whereas ectopic expression of HLH-1 could produce muscle-like cells in *lin-53*-depleted animals [11, 67]. The mammalian orthologs RBBP4 and RBBP7 function in several chromatin-regulating complexes with histone deacetylase (HDAC) activity and chemical HDAC inhibitors could phenocopy LIN-53 depletion [11]. In addition to histone acetylation, histone H3K27 methylation is also involved in cell-fate conversion. Inhibition of the LIN-53-containing PRC2 through RNAi against *mes-2* (EZH2), *mes-3* or *mes-6* (EED) abolished H3K27 methylation and similarly enabled CHE-1 to convert germ cells to ASE neurons [67]. These results are consistent with PRC2 preventing the germline from undergoing somatic differentiation programs.

Additional experiments suggest that PRC2, together with the H3K36 methyltransferase MES-4, also coordinately regulate global H3K27 and H3K36 methylation to promote gene expression patterns appropriate for germ cell fate. ChIP-seq experiments showed that MES-4 associates with and maintains methylation of germline-expressed genes through cell division and in a transcription-independent manner, a clear example of epigenetic inheritance [68]. In the absence of MES-4, PRC2-dependent H3K27 methylation was found to spread on germline genes [69]. Acquisition of H3K27me3 was found to be, at least partially, responsible for downregulation of germline-expressed genes on the autosomes in *mes-4* mutant germlines. Interestingly, antagonism between H3K27 and H3K36 methylation appears to be conserved across species [70, 71].

Reprogramming of somatic cells provides an alternative to the use of embryonic stem cells in regenerative medicine and is an area of intense research. In particular, erasing epigenetic marks laid down during differentiation is believed to be a critical step [72]. Interestingly, a naturally occurring transdifferentiation event in *C. elegans* was recently found to depend on chromatin modifiers. During larval development a particular rectal epithelial cell migrates anteriorly and changes cellular characteristics to become a motor neuron. Kagias *et al.* [73] used a candidate approach to screen for factors required for this process and identified several members of the Nanog and Oct4-associated deacetylase (NODE) HDAC complex, including SEM-4 (SALL4), EGL-27 (MTA1) and CEH-6 (OCT4). The effect of the NODE complex was reported to act at least partially via the Hox-like transcription factor EGL-5 but it is reasonable to expect that more genes are involved.

HISTONE MODIFICATIONS AND LONGEVITY

Caenorhabditis elegans has been a particularly powerful model system in aging research, paving the way for several instrumental findings [10]. In humans, epigenetic changes correlate with aging, both during normal physiological aging and in progeria syndromes [74], but the relevance is unknown. However, a genome-wide RNAi screen identified two *C. elegans* SET domain proteins, SET-9 and SET-15, whose inactivation increased lifespan [12], suggesting that chromatin modifications may control the aging process. More direct evidence was provided by a systematic analysis of putative and known methyl transferases and their interactors, which led to the identification of *ash-2*, *set-2*, *set-4* and *wdr-5* [75] (Figure 2). ASH-2 (ASH2L), SET-2 (SETD1) and WDR-5 (WDR5) are required for efficient methylation of H3K4 [34, 35] and their inhibition resulted in a ~20–30% extension of lifespan. Further support for a direct effect of H3K4 methylation on longevity was provided by inhibition of the

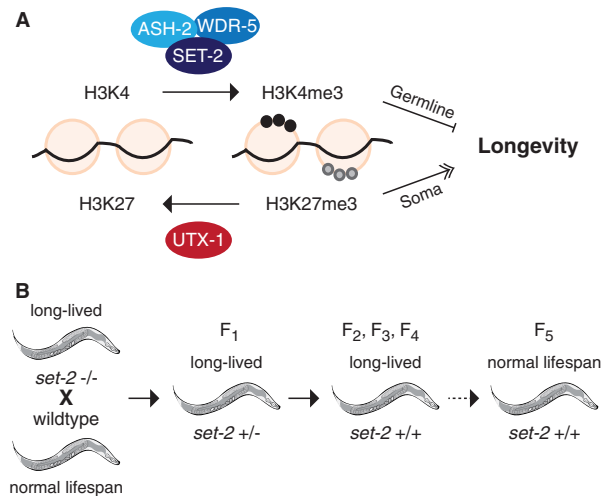


Figure 2: Effects of histone modifications on life span. **(A)** Both decreased deposition of H3K4me3 in *ash-2*, *set-2* or *wdr-5* mutants and increased levels of H3K27me3 in *utx-1* mutants promote longevity. The effect of the SET-2 methyltransferase complex on lifespan requires a functional germline, whereas demethylation by UTX-1 influences longevity via the soma. **(B)** Mutations in the SET-2 complex cause transgenerational inheritance of longevity. When homozygous *set-2* mutants (*set-2* $-/-$) are crossed with wild-type animals, the extended lifespan phenotype of *set-2* is inherited for up to four generations (F1–F4) despite these animals being genetically wild type (*set-2* $+/+$). By the fifth generation, normal lifespan is restored.

H3K4me3 demethylase RBP-2: *rbp-2* mutants have increased H3K4me3 levels and shortened lifespan [75]. Interestingly, ASH-2, SET-2 and WDR-5, but not SET-9 and SET-15, regulate longevity mainly via the germline and depend on production of mature egg, since their depletion did not extend lifespan of *fem-3* mutants, in which oogenesis is normal but fertilization is blocked [75]. The nature of the longevity-regulating signal from the germline to the soma is unknown but does not depend on the classical insulin/IGF-1 signaling pathway.

Strikingly, the positive effect of *ash-2*, *set-2* or *wdr-5* depletion on lifespan is stably inherited through several generations [76] (Figure 2). By crossing homozygous *ash-2*, *set-2* or *wdr-5* mutants with wild-type nematodes, Greer *et al.* could detect a significant longevity increase in genotypically homozygous wild-type animals up to four generations later. A crucial question remains concerning the mechanism behind the transgenerational inheritance: are specific epigenetic chromatin modifications inherited even in the presence of wild-type *ash-2*, *set-2* and *wdr-5* gene products or are secondary molecules responsible for the effect in subsequent generations? Global H3K4me3 levels are restored in genetically wild-type progeny that still are long-lived as their *ash-2*, *set-2* or *wdr-5* ancestors, but this does not exclude the possibility that modifications at specific loci are altered [76]. Indeed, active gene expression in parental adult germ cells has been found to correlate with more robust expression of the same gene in the somatic cells of offspring [77]. Several hundreds of genes are misexpressed in *wdr-5* mutants suggesting that the inherited signal may be a particular (combination of) protein and/or RNA and represent targets for further studies [76]. Considering the results described in a previous section, proteins involved in small RNA metabolism, including argonautes, are interesting candidates.

While levels of H3K4me3, typically associated with active chromatin, inversely correlate with longevity the opposite has been reported for H3K27me3, which is enriched in silenced chromatin. Inhibition of the demethylase UTX-1 (KDM6A) increased H3K27me3 levels and prolonged lifespan by ~17–30% [78, 79]. In contrast to *ash-2* and *set-2*, the longevity increase upon *utx-1* depletion is germline independent, but requires the insulin/IGF-1 signaling pathway. At least part of *utx-1* function may be at the level of regulation of H3K27 methylation of genes implicated in insulin/IGF-1 signaling, such

as *daf-2*, *akt-1* and *akt-2* [78]. Both in old nematodes [79] and in cells from human progeria patients [80] H3K27me3 global level decreases, suggesting that age-dependent increase in UTX-1/KDM6A activity may play a conserved role during aging [78].

CONCLUSION AND FUTURE PERSPECTIVES

Research on *C. elegans* has convincingly shown that epigenetic modifications have profound effects on development by controlling chromosome organization, gene expression and cell identity. Most studies have focused on phenotypes in specific tissues, but because chromatin modifiers are generally ubiquitously expressed, determining what brings about tissue specificity remains a major challenge. Possibly, a complicated interplay between broadly expressed chromatin modifiers and more restricted transcription factors and non-coding RNAs may control spatial and temporal effects. Global profiles of histone modifications have been obtained for embryos and whole animals, but tissue-specific maps are required to decipher the link between epigenetic landscapes and cell identity. Relating this information to the physical organization of the nucleus will most likely provide a more complete picture of the dynamics that take place as germ cells produce zygotes and these develop to adult animals.

In addition to understanding basic concepts of biology, the knowledge we are accumulating may generate important benefits to human health via regenerative medicine. Stem cells have the potential to repopulate damaged organs or substitute deficient cells, but establishing induced pluripotent stem cells as well as differentiation of induced pluripotent or embryonic stem cells to specific cell lineages involves reprogramming of epigenetic marks. Identification of molecular players and signaling pathways that control naturally occurring epigenetic changes may help developing efficient and safe *in vitro* protocols for stem cell manipulation.

Key Points

- SET-25 and MET-2, two H3K9 methyltransferases, are responsible for silencing and anchoring of heterochromatin to the nuclear periphery.
- Mutations and naturally occurring changes in histone modifications can switch cell identity from germ cell to differentiated cell or vice versa or induce transdifferentiation of somatic cells.

- *Caenorhabditis elegans* Argonaute proteins participate in multiple processes ranging from RNAi to transcriptional silencing to chromosome segregation.
- Alterations of gene expression due to chromatin modifications can be inherited for several generations; such alterations may reflect developmental decisions or genetic backgrounds.

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