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Review

Activity and Silencing of Transposable Elements in *C. elegans*

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Abstract: Since the discovery of transposable elements (TEs) in maize in the 1940s by Barbara McClintock transposable elements have been described as junk, as selfish elements with no benefit to the host, and more recently as major determinants of genome structure and genome evolution. TEs are DNA sequences that are capable of moving to new sites in the genome and making additional copies of themselves while doing so. To limit the propagation of TEs, host silencing mechanisms are directed at transposon-encoded genes that are required for mobilization. The mutagenic properties of TEs, the potential of TEs to form new genes and affect gene expression, together with the host silencing mechanisms, shape eukaryotic genomes and drive genome evolution. While TEs constitute more than half of the genome in many higher eukaryotes, transposable elements in the nematode *C. elegans* form a relatively small proportion of the genome (approximately 15%). Genetic studies of transposon silencing, and the discovery of RNA interference (RNAi) in *C. elegans*, propelled *Caenorhabditis elegans* (*C. elegans*) to the forefront of studies of RNA-based mechanisms that silence TEs. Here, I will review the transposable elements that are present and active in the *C. elegans* genome, and the host defense mechanisms that silence these elements.

Keywords: transposable element (TE); RNA interference (RNAi); transposon; retrotransposon; silencing; small interfering RNA (siRNA); *Caenorhabditis elegans* (*C. elegans*); Piwi-interacting RNA (piRNA)

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1. Introduction

Transposable elements (TEs) [1], also called "jumping genes", are a widespread class of mobile genetic elements that can move and replicate within a genome. Almost all eukaryotic genomes contain TEs. TEs can make up large proportions of eukaryotic genomes; around half the human genome [2] and about 15% of the *Caenorhabditis elegans* (*C. elegans*) genome [3] are transposon sequences. The proportion of TEs in the genome can differ vastly in organisms of similar evolutionary complexity, which largely explains the vast differences in genome size between such organisms.

In addition to having profound effects on genome size, TEs are mutagenic, cause genome instability and are targeted by silencing mechanisms. Because TEs insert into new sites into the genome, they may disrupt genes. When TEs excise, they can leave footprints or trigger the deletion of flanking sequences as a result of double-stranded break repairs. TEs can also promote recombination and chromosomal rearrangements because of their repetitive nature. All these types of sequence changes may cause disease. On the other hand, transposons drive genome evolution and may be beneficial in adaptation to changed conditions. Regulation of transposon silencing mechanisms by environmental stresses could allow for transposon activity under abrupt environmental changes, and therefore potentially mediate adaptation [4].

TEs also affect genomes through the chromatin-based host silencing mechanisms directed at transposons. TE silencing mechanisms include chromatin modification, repressive complexes, RNA-directed DNA methylation, RNA interference (RNAi) and sequence-specific transcription factors. Constitutive heterochromatin in the centromeric and pericentromeric regions of human chromosomes is highly enriched in repeat elements such as TEs [5]. Similarly, the distal arms of the autosomal chromosomes of the holocentric

animal *C. elegans* are enriched with heterochromatic proteins and marks, and TEs [6–8]. In addition to silencing of TE transcription in heterochromatin, homologous recombination is also suppressed. This lack of homologous recombination is thought to contribute to the vast numbers of repetitive sequences accumulating in heterochromatin, as these elements cannot be eliminated by homologous recombination [9].

In addition to generating sequence variation that may disrupt genes, TEs also contribute to the creation of new genes. As a result of transposition into genes, host protein domains can fuse with transposon-encoded protein domains, which can lead to novel host functions. This process is called co-option or domestication. Transposons can also provide new regulatory sequences to host genes, since TEs contain promoters, polyadenylation signals and transcription factor binding sites.

New TEs can enter host genomes through horizontal gene transfer, the non-sexual movement of genetic information between genomes. The horizontal gene transfer of TEs leads to phylogenetic incongruence, a strong conflict between the phylogenies of the transposon and the organism. The horizontal gene transfer of TEs has been well documented in flies [10] and has also recently been demonstrated in large-scale studies of vertebrate genomes [11].

The nematode *C. elegans* was chosen as an animal model system to study development and behavior by Sydney Brenner in 1965 [12]. *C. elegans* was the first multicellular organism for which the genome was completely sequenced and published, in 1998 [13]. Genetic studies in the 1980s identified natural isolates of *C. elegans*, including one from Bergerac (France) that in comparison to the "wild type", or reference isolate, from Bristol (England), showed a repeated sequence that was polymorphic and displayed high rates of spontaneous mutations [14–16]. These observations had several implications for *C. elegans* research in general and for the understanding of transposon silencing in particular. Tc1 was identified as an active DNA transposon in *C. elegans*. Genetic analysis showed that *mutator* loci in the genome were responsible for the *mutator* phenotype in the Bergerac strain, indicating the possible existence of a silencing mechanism in the Bristol strain. A practical benefit of these discoveries was the development of transposon tagging using Tc1 to rapidly identify mutated genes in forward genetic screens, and the development of transposon insertion libraries that were used to find knock-out mutations in genes of interest.

2. Transposable Elements in the *C. elegans* Genome

Eukaryotic transposable elements are classified into two classes: Class I—retrotransposons and Class II—DNA transposons [17]. Retrotransposons transpose via an RNA intermediate that is reverse transcribed into a dsDNA intermediate, which is integrated into the genome (Figure 1). The original copy remains at its genomic location, and therefore these TEs move via a "copy-and-paste" mechanism. This is in contrast to DNA transposons, which typically move via a "cut-and-paste" mechanism (Figure 1), although this does not apply to all types of DNA transposons. Typically, a DNA transposon is cut out by a transposase protein and integrates into another site in the genome. Within the two classes of TEs, transposons are further subdivided into subclasses and superfamilies based on their mechanisms of replication and integration and their phylogenetic relationships [18]. Finally, families of transposons are defined by DNA sequence similarity of 80% in at least 80% of the aligned sequence (either the coding region or terminal repeats) with a minimum of 80 bp. Multiple transposon copies of one family can be aligned to generate a consensus sequence that represents the active ancestral TE. Many of the TEs present in the *C. elegans* genome are inactive because of mutations in the open reading frames of the transposon-encoded proteins required for mobilization [19], or because of mutations in the terminal inverted repeats (TIRs) or long terminal repeats (LTRs). However, since the transposon-encoded proteins can act in trans, a protein encoded by another element of the same family may be able to mobilize a transposon that no longer encodes the protein(s) required for transposon mobility. The most extreme example of this phenomenon is represented by miniature inverted-repeat transposable elements (MITEs), which only consist of the inverted repeats

of the parental transposon, but that can be mobilized in trans by a transposase protein encoded by a full-length element [20]. These non-autonomous transposons are subfamilies of the full-length transposon family that harbors the transposon-mobility genes with which they share an inverted repeat sequence that is recognized by the transposase.

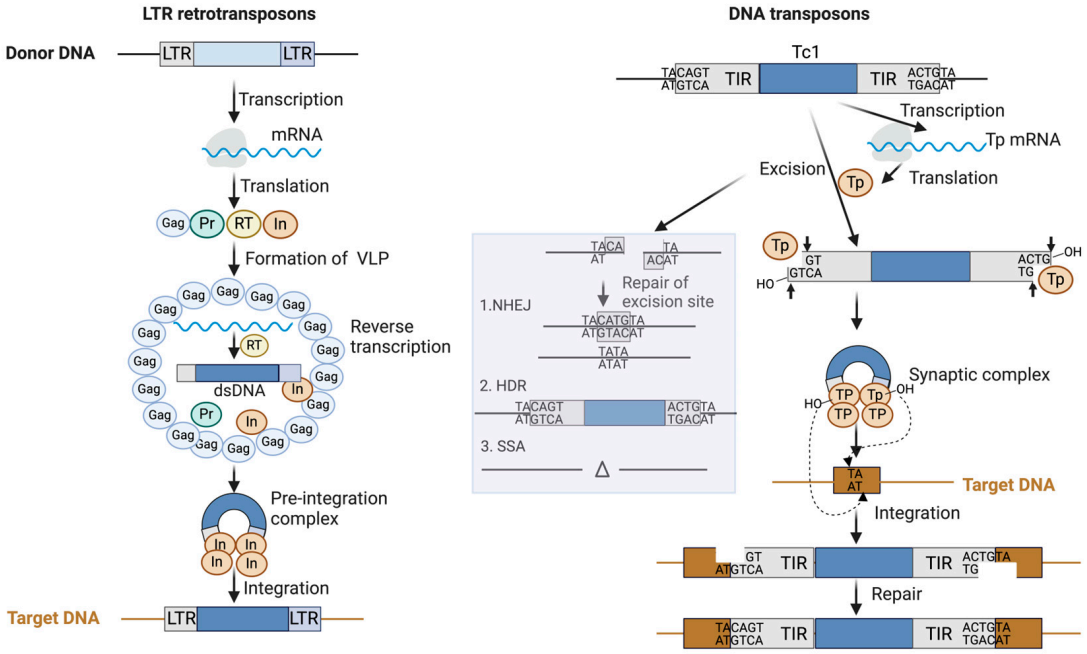

Figure 1. Transposition mechanisms of LTR retrotransposons and DNA transposons (Tc1). LTR retrotransposons move through an RNA intermediate that is reverse-transcribed in virus-like particles (VLPs). DNA transposons move via a copy-and-paste mechanism mediated by the transposon-encoded transposase (Tp) protein. The outcomes of repair of the excision site by three possible repair pathways (NHEJ, HDR and SSA) are shown. Created with BioRender.com.

The known active transposons in *C. elegans* are members of Class II DNA transposons of the Tc1/mariner superfamily, named after the *C. elegans* Tc1 transposon (Table 1). The Tc1/mariner superfamily is the most widespread TE family among eukaryotes. Tc1 was identified in the 1980s as a repeated sequence that was polymorphic between two natural isolates of *C. elegans* [14–16]. This polymorphic sequence was shown to be mobile in studies of spontaneous mutations of two muscle genes that are reversible [21], and thus represented a transposon. Research into the underlying genetic loci that allow for mobilization of Tc1 in the germline [22], led to the discovery that RNAi silences transposable elements [23,24]. We now know that RNAi is an evolutionarily deeply conserved mechanism to silence viruses and TEs.

The *C. elegans* reference strain most widely used in the laboratory (Bristol) shows very little transposition activity in the germline, although transposition does occur in somatic cells [25]. However, in some other isolates of *C. elegans*, such as the Bergerac strain, transposons do jump in germline cells. These isolates can have highly increased copy numbers of transposons, with up to 748 Tc1 elements in the Bergerac strain [26]. The most active transposons identified in such strains, as well as in strains with *mutator* mutations isolated in screens for transposon activation, are the related transposons Tc1 and Tc3 [27], which are present in 32 and 22 copies, respectively, in the Bristol strain genome (Table 1). Tc1/mariner transposons move via a cut-and-paste mechanism described below. Whereas germline transposition is suppressed in the Bristol strain, Tc1 and Tc3 are active in the germlines of *mutator* mutants such as *mut-2/rde-3* and *mut-7* (described below) [15], and Tc1 but not Tc3 is specifically mobilized in the germline of the Bergerac strain and other uncloned *mutator* mutants [27,28]. Tc7 is a non-autonomous element related to Tc1, that is active in somatic cells of *mut-6* and *mut-7* mutants [29]. Also active in *mutator* mutants

mut-2/*rde-3* and *mut-7* are the related Tc1/mariner transposons Tc4/Tc4v and Tc5 [30–32]. Tc2 [33] is a pogo transposon that is related to Tc1/mariner transposons but may form a separate superfamily [34,35]. Tc2 is not active in *mutator* strains but is active in strains derived from the Bergerac and Bristol crosses.

Table 1. Active transposons in the *C. elegans* genome.

Element	Class	Order	Superfamily	Family	Copy Number	Length (bp)	Catalytic Motif	IR/TIR Length (bp)	Target Site (Duplicaton)
Cer1	Class I	LTR	Gypsy	Gypsy	1	8865	DDE	492	
Tc1	Class II	TIR	Tc1/mariner	Tc1	32	1611	DD34E	54	TA (TA-TA)
Tc2	Class II	TIR	Tc1/mariner-Tc2/pogo group	Pogo	4	2074	DD35D	24	TA (TA-TA)
Tc3	Class II	TIR	Tc1/mariner	Tc1	22	2335	DD34E	462	TA (TA-TA)
Tc4/Tc4v	Class II	TIR	Tc1/mariner-Tc4 group	Tc4	10	1605/3483	DD37D	774	CTNAG (TNA-TNA)
Tc5	Class II	TIR	Tc1/mariner-Tc4 group	Tc4	4	3171	DD37D	491	CTNAG (TNA-TNA)
Tc7 (Tc1 MITE)	Class II	TIR	Tc1/mariner	Tc1	11	921	n/a	345	TA (TA-TA)

Shown are the transposons in the *C. elegans* genome that have been shown to be active, with the exception of the Cer1 retrotransposon, which generates VLPs but for which no new insertions have been found. Indicated are the classifications of the transposons and their characteristics such as full-length copy number, length of the transposon, the catalytic motif, length of the terminal repeats and the sequence of the target site and of the target site duplication after insertion.

Only for these six DNA transposons is there evidence of active transposition in *C. elegans*; new insertions have been found in Bristol strain mutants and in *C. elegans* natural isolates. Other than these six elements, the *C. elegans* genome contains 752 TEs from 224 families [3]. These TEs include numerous additional Class II elements, including elements from the Tc1/mariner, helitron, *mutator*, CACTA and maverick superfamilies. Whereas the human genome contains a large number of Class I elements covering over 40% of the genome [36], the *C. elegans* genome contains relatively few Class I TEs. There are 20 full-length copies of LTR retrotransposons of the gypsy [37] and bel-pao superfamilies [38–40], as well as two SINE elements and LINE elements (non-LTR retrotransposons) of the RTE [41] and R2 [42] superfamilies. LTR retrotransposons structurally and mechanistically resemble retroviruses but lack the envelope proteins required for the extracellular phase of the retroviral life cycle. Some *C. elegans* LTR retrotransposons encode for envelope proteins and could therefore be endogenous retroviruses (ERVs). Whereas LTR retrotransposons typically only encode the gag, pol and possibly env polyproteins, several *C. elegans* LTR retrotransposons encode for additional proteins [43,44].

Some of the 752 TEs may be active under conditions of environmental stress or in mutant backgrounds that have not been studied. However, only a limited number of transposon-encoded genes in the genome appear to have the potential to be mobile [19]. Recent mobility of some of these elements in other genetic backgrounds has been deduced from studies of naturally occurring polymorphisms and genome sequencing of *C. elegans* natural isolates [3,45]. A well-studied example of this is the Gypsy superfamily LTR retrotransposon Cer1 (Table 1). The Bristol strain has a single insertion of Cer1 in its genome, inactivating a mucin gene [45]. This insertion is absent in two thirds of the natural isolates, but half of these do have Cer1 elsewhere in their genome. Cer1 produces virus-like particles (VLPs) in the Bristol strain [46] but no new insertions of Cer1 have been observed in this strain. In a large-scale genome sequencing study of transposons in *C. elegans* natural isolates, over 60% of TEs shared sites in all natural isolates, indicating that

these are inactive. Some of the 241 TE sites that were not present in all natural isolates may represent transposons that are active under specific conditions or genetic backgrounds.

3. Mechanisms of Transposition in *C. elegans*

Biochemical studies and sequence analysis elucidated the mechanism of Tc1 and Tc3 transposition [47,48] (Figure 1). Tc1 contains one gene, encoding the Tc1 transposase, flanked by terminal inverted repeats (TIRs). Transposase is the only protein required for movement and can even catalyze transposition in cell-free assays, which is likely to account for the successful spread of Tc1/mariner elements between widely diverged taxa. The transposase protein contains a bipartite N-terminal DNA-binding domain [49–51] and a C-terminal endonuclease domain that contains a catalytic triad of acidic residues (DDE/D) found in transposases and retroviral integrases (Table 1).

The N-terminal domain of Tc1 transposase binds to specific sequences within the TIRs. The transposase hydrolyzes the phosphodiester bonds at the termini TIRs to cut the transposon out of its genomic location and form a synaptic complex. At the target site, which is always a TA dinucleotide, the transposase catalyzes a transesterification reaction to insert the transposon into the target site. The staggered cuts result in duplication of the TA target sequence. Host DNA double-stranded break repair mechanisms repair the transposon excision site via non-homologous end-joining (NHEJ), via homology-directed repair (HDR) (homologous recombination), or via single-strand annealing (SSA) (Figure 1). Depending on the specific repair pathway, a short footprint is left at the excision site (NHEJ), a larger deletion around the excision site is created (SSA), or the site is repaired via homologous recombination using sister chromatid or other homologous copies, retaining the transposon at the original location. The latter mechanism results in an increase in transposon copy numbers. Homologous recombination can also contribute to the rapid evolution of TEs by the creation of internally deleted transposon copies via strand slippage or template switching [52]. Which repair pathway is used is in part dependent on the tissue; repair by homologous recombination is most prevalent in the germline of *C. elegans*, while NHEJ and SSA are more active in somatic cells. However, all three pathways are used for repair after excision of Tc1 in the germline of *C. elegans*. Sequence exchange between Tc1 elements demonstrated the use of HDR [52], whereas footprints found after Tc1 excisions are a consequence of NHEJ [53]. Finally, deletions of sequences flanking the Tc1 excision site are a consequence of SSA repair [54].

Although Tc1 and Tc3 insert into TA dinucleotides, the sequences immediately flanking the TA affect the target choice [55–57]. On the level of the chromosomes, the Tc1 elements jump from and into all chromosomes, but reside in regions of repressed chromatin and are underrepresented in domains with highly active chromatin marks [26]. In general, DNA transposons are found on the autosomal chromosome arms of *C. elegans* [3,57].

LTR retrotransposons like Cer1 transpose via an RNA intermediate (Figure 1). After transcription, some of the retrotransposon transcripts are used for the translation of the LTR retrotransposon gag and pol genes. The Gag proteins form virus-like particles (VLPs) that contain the pol proteins (protease, integrase, and reverse transcriptase) and dimeric retrotransposon genomic RNA (gRNA) transcripts that interact with the Gag protein via CCHC Zn fingers in the nucleocapsid domain. In the VLPs, the RNA is reverse transcribed to form double-stranded complementary DNA (cDNA), which forms a complex with the integrase. This pre-integration complex moves to a new genomic location and the integrase cleaves the genomic DNA via a mechanism similar to the related protein Tc1 transposase and integrates the LTR retrotransposon DNA. Like DNA transposons, LTR retrotransposons are found on chromosome arms [40]. Although Cer1 produces VLPs, new insertions of Cer1 have not been identified in the reference strain, suggesting that Cer1 may not be active.

4. Silencing of Transposable Elements

The observation that Tc1 elements are active in the germline of the Bergerac isolate, but not in the reference strain and most natural isolates, indicated that a silencing mechanism

exists in the germline of the reference strain. Although the loci from the Bergerac strain that allow for Tc1 hopping have not been identified, genetic screens for *mutator* mutants in which Tc1 and/or Tc3 are desilenced in the reference strain, have uncovered core factors in transposon silencing pathways. At the same time, similar screens for RNAi-defective mutants identified an overlapping set of genes indicative of the role of RNAi in silencing transposable elements.

In most animals, Piwi proteins together with Piwi-interacting RNAs (piRNAs) silence TEs in the germline. In *C. elegans* Piwi and piRNAs also act in transposon silencing and are required for silencing of Tc3 in the germline (Figure 2) [58]. Most of the ~15,000 *C. elegans* piRNAs are transcribed from piRNA genes located in two large clusters on chromosome IV [58]. These clusters contain TEs that have been inserted behind piRNA promoters. After the processing of a piRNA precursor into a mature 21 nt RNA, the piRNA associates with the *C. elegans* Piwi protein PRG-1 (reviewed in [59]). The PRG-1-piRNA complex sequence-specifically recognizes its target (transposon) mRNA through base pairing. PRG-1 localizes predominantly to peri-nuclear P granules, but it may also act elsewhere in the cytoplasm. Base pairing between piRNA and target mRNA accommodates multiple mismatches [60]. The large number of piRNAs in combination with such relaxed targeting allows for the silencing of newly acquired transposons or rapidly diverging elements without a need to produce new small RNAs specific to the transposon [61]. Conversely, many non-transposon genes are targeted by piRNAs. To counteract the silencing of self-genes and to promote their expression in the germline, the CSR-1 Argonaute, in association with endogenous small interfering RNA (siRNAs), protects these self-genes against piRNA-mediated silencing.

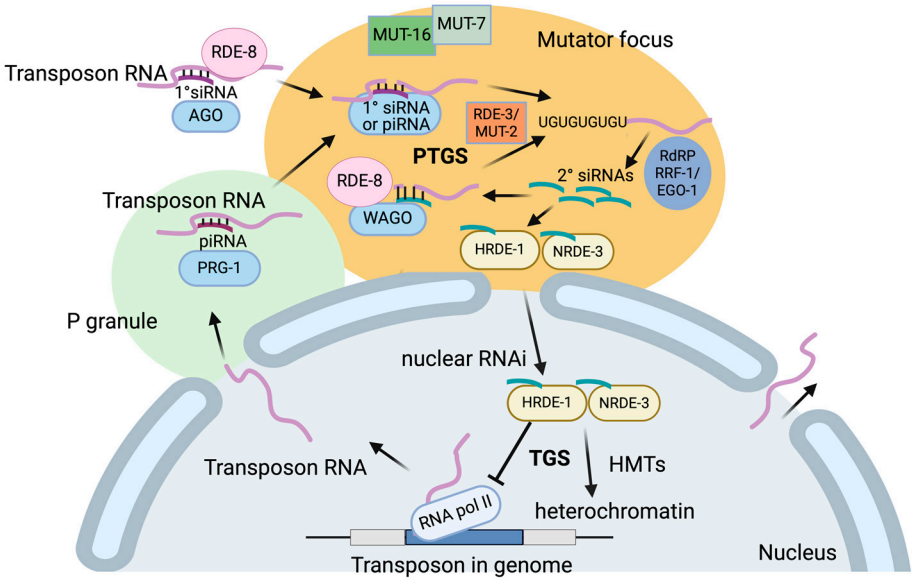

Figure 2. A simplified model of RNAi-mediated transposon silencing in *C. elegans*. Transposon RNA is targeted by piwi-interacting RNA (piRNA) loaded onto PRG-1. Cleavage of transposon RNA by RDE-8 or its paralogs triggers the addition of a poly(UG) tail by RDE-3. The poly(UG) tail recruits an RNA-dependent polymerase to generate secondary small interfering RNAs (siRNAs) that trigger post-transcriptional gene silencing (PTGS) and sustained siRNA amplification bound to WAGOs, and transcriptional gene silencing through the nuclear Argonautes HRDE-1 and NRDE-3.

After target recognition by the piRNA, secondary siRNA generation is triggered in peri-nuclear phase-separated *mutator* foci [62] (Figure 2). Loss of components of *mutator* foci, such as the exonuclease MUT-7 and the scaffold protein MUT-16, results in a loss of transposon silencing and the activation of transposons in the germline [23,63]. Secondary siRNA generation may be initiated by slicing or cleavage of the mRNA by the endonuclease RDE-8. The poly(UG)-polymerase RDE-3 (also known as *mut*-2) adds a poly(UG) tail to the 3′end of the target mRNA fragment [64]. The pUG tail recruits an RNA-dependent

RNA polymerase (RdRP) that generates secondary siRNAs using the transposon RNA as a template [65]. Four distinct functional Argonaute (AGO) proteins can be loaded with these transposon siRNAs [66]: WAGO proteins that act in post-transcriptional gene silencing (PTGS) and promote continued siRNA generation by RDE-3 and the RdRP, and the nuclear Argonaute protein HRDE-1 [67], which in a complex with siRNAs and other nuclear RNAi factors directs transcriptional gene silencing (TGS) of the transposon. Transcriptional gene silencing is mediated by a direct inhibition of RNA polymerase II transcribing the target transposon, and by the deposition of repressive chromatin marks at histone H3 lysine 9 (H3K9me3) [68,69], histone H3 lysine 27 (H3K27me3) [70], and histone H3 lysine 23 (H3K23me3) [71], directed by the nuclear RNAi machinery. HRDE-1 induces heterochromatin formation and chromatin compaction [72,73]. Transcription within heterochromatin generates additional small RNAs that are loaded onto HRDE-1 to promote further small RNA amplification in *mutator* foci [72].

Regions with heterochromatin marks such as the presence of HPL-2, one of two *C. elegans* heterochromatin protein 1 orthologs, and histone H3K9me2 and H3K9me3 [8,57,74], are enriched with TEs and other repeats in *C. elegans*. In addition to HPL-2, heterochromatin proteins MET-2/SETDB1, LIN-61, LIN-13 and LET-418/Mi-2 are enriched at repetitive elements and are required for transcriptional silencing of a subset of TEs [7]. Many of the TEs suppressed by the heterochromatin proteins are silenced by piRNAs [8].

The Polycomb Repressive Complex (PRC2) is an evolutionary conserved protein complex in metazoans that directs methylation of histone H3 on lysine 27 (H3K27), a hallmark of facultative heterochromatin. PRC2 functions in the repression of gene expression to maintain correct patterns of gene expression, but also acts in the silencing of TEs in divergent eukaryotes, suggesting that this is an ancestral function of the PRC2 complex [75,76]. *C. elegans* contains a diverged PRC2 core [77] that mediates H3K27 di- and tri-methylation. These marks are distributed more broadly over the genome than the H3K9me2 and H3K9me3 marks associated with silenced TEs, and they are particularly widespread on the X chromosome [8,78]. The *C. elegans* PRC2 genes (*mes-2/EZH2, mes-3* and *mes-6/EED*) are required for germline development and male tail development but have not been implicated in transposon silencing. However, since H3K27me3 marks do partially colocalize with H3K9me3 on the TE-rich distal chromosome arms and can be deposited through the nuclear RNAi pathway [70], PRC2 and H3K27 methylation may play a role in the silencing of TEs in *C. elegans*.

DNA methylation of the C5 position of cytosine (5mC) is a common epigenetic mark in vertebrates, in plants, and in some but not all invertebrates and fungi. In plants, small RNAs direct DNA methylation to silence TEs, and DNA methylation also represses TEs in mammals [79]. *C. elegans* lacks detectable 5mC DNA methylation and the *C. elegans* genome does not encode the DNMT1 and DNMT3 DNA methyltransferases that methylate the C5 position of cytosine [80]. Thus, TEs in *C. elegans* are not regulated by 5mC DNA methylation.

The nuclear RNAi machinery, including HRDE-1, mediates trans-generational silencing of transposons, which, once established, does not require the piRNA trigger [81]. Recent reviews describe trans-generational RNAi in detail [82–84]. The heritable nature of RNAi-mediated transposon silencing allows for an immediate silencing response to invading TEs that have sequence similarity to previously encountered TEs, reminiscent of the adaptive immune system in humans.

Some transposons are not silenced by piRNAs. For example, a set of non-conserved genes, which includes several retrotransposons, is silenced through primary siRNAs (as opposed to piRNAs) that are loaded onto the Argonaute ERGO-1-triggering secondary siRNA generation and nuclear RNAi [85]. It is likely that other mechanisms of transposon recognition upstream of the secondary siRNA generation exist.

One feature of transposon RNA that may be recognized by silencing pathways is the inefficiency or absence of splicing of transposon RNA because of poor splice sites, lack of introns, or other suboptimal features of transposon pre-mRNAs [86]. The Tc1 transposase gene contains an intron but is only spliced in a small fraction of transcripts [87]. The

retention of Tc1 transcripts on spliceosomes suggests that defects in splicing could trigger silencing [86]. Indeed, the presence of bona fide introns protects *C. elegans* genes against silencing [88]. EMB-4, a homolog of the intron-binding helicase Aquarius, interacts with HRDE-1 and initiates silencing of an overlapping set of TEs, but is only required to silence intron-containing target RNAs [89]. Thus, EMB-4 is important to overcome the protection against silencing of piRNA targets that do contain introns.

Another feature of most DNA transposons is the presence of TIRs that can form dsRNA structures. These structures are not only targets of the RNAi enzyme Dicer but can also be targeted by other dsRNA-binding and dsRNA-modifying proteins such as the dsRNA receptor RIG-I and the ADAR enzymes that edit dsRNA. There are no reports of a role for ADARs in the silencing of Tc1 and Tc3 transposons, but ADARs do act on small palindromic repeat elements such as Tc7 [43]. ADAR editing of these repeats competes with the silencing of these dsRNAs by the RNAi pathway. It is unknown if and how such RNA editing would affect the mobilization of these palindromic repeat elements.

An alternative way to silence the effects of transposable elements is to ensure that even when a transposon is inserted into a gene, excision of the transposable element does not inactivate the gene. A high proportion of Tc1 insertions into genes are silent. The silent nature of Tc1 insertions can be the result of frequent excisions that leave minimal footprints that retain the reading frame. Alternatively, the open reading frame can be restored by the use of these footprints as alternative splice sites [90]. Even if Tc1 is not excised from the DNA, it is spliced out in a high proportion of mRNAs [91].

5. Other Mechanisms of Transposon Regulation: Adaptation and Domestication

A proposed benefit for the host of the presence of TEs in the genome is the potential to adapt to unfavorable or changed conditions by desilencing TEs to generate random mutations. In relation to transposon silencing in *C. elegans*, the most studied stresses are growth at elevated temperatures and acute heat stress. The latter stress induces DNA damage and Tc1 mobilization in the male germline but not the female germline. Tc3 is not mobilized by heat stress, again indicating that these transposons are differentially regulated [92]. Longer-term exposure to elevated temperatures that are not lethal to wild-type animals, results in sterility and elevated transposition rates in *mutator* mutants [23]. These reports show that heat stress does result in an increase in transposon mobilization. Many small RNA pathway mutants, including *mutator* mutants display a progressive decline in fertility, a mortal germline, over generations, that for some mutants is dependent on elevated temperature. This sterility is not caused by the accumulation of DNA damage due to an increase in TE mobilization but is more likely to be a consequence of a broader mis-regulation of gene expression in the germline [93].

Evidence of the potentially beneficial nature of TEs to the host is found in the domestication or co-option of TEs. In *C. elegans*, the Cer1 Gag protein and VLPs act in the horizontal transfer of the memory of pathogen avoidance to naïve animals [94]. Co-option of transposons as regulatory sequences in *C. elegans* is widespread; a third of the germline-specific promoters have been co-opted from two miniature inverted-repeat transposable elements (MITEs) [95]. A protein with DNA-binding domains related to a transposase protein regulates these promoters. Whereas the transposons have become part of host genes in the case of the above-mentioned MITEs, transposons can also regulate host genes in their vicinity. Over half of the heat shock elements (HSEs) are found within Helitron DNA transposons in *C. elegans* [96]. HSEs are bound by Heat Shock Factor 1 (HSF-1) in response to elevated temperatures to drive the transcription of genes that reduce the proteotoxic effects of heat stress. The binding of HSF-1 to Helitron HSEs drives the transcription of neighboring genes upon heat stress. The mobility of Helitron transposons thus mediates the incorporation of new genes into the HSR pathway, driving evolution through changes in gene regulation.

6. Conclusions and Future Directions

The breadth of this review reflects the wide-ranging effects of TEs on the *C. elegans* genome, from the mutagenic properties of TEs to the complex and intricate silencing mechanisms that the host mounts to silence transposable elements.

Transposon research in *C. elegans* started with investigations of a natural isolate in which the Tc1 transposon hops. While *C. elegans* research into RNAi and transposon silencing has contributed a wealth of knowledge and understanding of these processes, it is still unknown why Tc1 is desilenced in this Bergerac natural isolate. Using CRISPR-Cas, transposon-specific silencing pathways could be uncovered that may be independent of the known RNAi factors. It will be of particular interest to determine if there are mechanisms of transposon recognition other than piRNA complementarity, especially given that not all transposons appear to be silenced through Piwi and piRNAs. It is also unclear if TE silencing is affected by environmental stresses other than heat stress and how TEs contribute to the adaptation of the host to environmental stresses.

The large number of *C. elegans* natural isolates and related nematodes are a valuable resource for studying additional potentially active transposons and silencing mechanisms, especially given the rapid evolution of transposons and silencing pathways. *Caenorhabditis inopinata* is a nematode related to *C. elegans* that has undergone a massive expansion in TEs, including Tc1/mariner transposons and LTR retrotransposons. It will be of interest to explore the genetic differences that could underlie this burst in transposon activity. Given that *C. inopinata* has lost its ERGO-1 pathway, this pathway may play a major role in curtailing transposon proliferation. Finally, whereas most research has focused on transposons that are active, transposons that do not appear active may have been co-opted and fulfill new functions in the host, such as the retrotransposon Cer1 and the Helitron transposons, and therefore should not be discarded as junk.

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