Biol. Cell (2018) **110,** 1–8 DOI: 10.1111/boc.201800031

Review

Distinct nuclear and cytoplasmic machineries cooperatively promote the inheritance of RNAi in Caenorhabditis *elegans*

Fei Xu, Shouhong Guang[1] and Xuezhu Feng[1]

Hefei National Laboratory for Physical Sciences at Microscale, School of Life Sciences, University of Science and Technology of China, Hefei, Anhui 230027, P. R. China

Epigenetic information can be inherited over multiple generations, which is termed as transgenerational epigenetic inheritance (TEI). Although the mechanism(s) of TEI remains poorly understood, noncoding RNAs have been demonstrated to play important roles in TEI. In many eukaryotes, double-stranded RNA (dsRNA) triggers the silencing of cellular nucleic acids that exhibit sequence homology to the dsRNA via a process termed RNA interference (RNAi). In Caenorhabditis *elegans*, dsRNA-directed gene silencing is heritable and can persist for a number of generations after its initial induction. During the process, small RNAs and the RNAi machinery mediate the initiation, transmission and re-establishment of the gene silencing state. In this review, we summarise our current understanding of the underlying mechanism(s) of transgenerational inheritance of RNAi in *C. elegans* and propose that multiple RNAi machineries may act cooperatively to promote TEI.

Twenty years ago, it was demonstrated that dsRNAs could silence gene expression with cognate nucleic acid sequences in Caenorhabditis *elegans* by an injection of double-stranded RNA (dsRNA) [Fire et al., 1998]. Later, small regulatory RNAs were identified to play essential roles in gene silencing events. Small regulatory RNAs regulate gene expression, developmental timing, antiviral defence, and genome integrity *via* a process termed RNA interference (RNAi) [Billi et al., 2014; Hannon et al., 2006]. Small RNAs direct the RNAi and chromatin modification machineries to targeted nucleic acids and silence gene expression via a number of mechanisms, including inhibiting transcription and translation, destabilising mRNAs and eliciting epigenetic alterations. Notably, these acquired small RNAs and epigenetic changes can persist and be transmitted from parents to offspring for multiple generations. Transgenerational inheritance of RNAi allows organisms to remember their previous exposure to genome parasites, transmit the experience to their descendants, and may provide evolutional advantages to enable the selection of physiologically beneficial traits (for a comprehensive review, please refer to Rechavi and Lev, 2017].

The mechanisms of transgenerational inheritance of RNAi are widely studied in *C. elegans*, and they have been extensively reviewed in many recent publications (reviewed by Feng and Guang, 2013; Heard and Martienssen, 2014; Lim and Brunet, 2013; Minkina and Hunter, 2018; Miska and Ferguson-Smith, 2016; Rechavi and Lev, 2017). Both exogenously derived siRNAs (exo-siRNAs) and endogenous small RNAs, such as endo-siRNAs and PIWI-interacting small RNAs (piRNAs), can trigger heritable RNAi. The RNAi-mediated silencing effect can be transmitted *via* parental gametes [Alcazar et al., 2008; Grishok et al., 2000]. In addition, the maintenance of silencing requires the expression of targeted genes [Minkina and Hunter, 2017].

[1]To whom correspondence should be addressed (email: sguang@ustc.edu.cn and fengxz@ustc.edu.cn)
Key words: Gene silencing, Inheritance, RNAi, Small RNA.
Abbreviations: dsRNA, double-stranded RNA; NRDE, nuclear RNAi defective; piRNAs, PIWI-interacting small RNAs; RNAi, RNA interference; TEI, transgenerational epigenetic inheritance.

Although many lines of evidence have demonstrated that the RNAi machinery and small RNAs are involved in the inheritance of RNAi, many questions remain unanswered regarding mechanisms and functions. For example, under what circumstances can environmental stress initiate the generation of heritable small RNAs? What is the precise nature of the trait-specific information that is transmitted from the parents? What is the mechanism of the re-establishment of gene silencing in progeny? In this review, we will summarise some of the latest research on the mechanism of RNAi inheritance and propose that multiple RNAi machineries may act cooperatively to promote transgenerational RNAi inheritance.

The nuclear RNAi pathway promotes the transgenerational inheritance of RNAi

dsRNAs silence nuclear-localised RNAs in *C. elegans* through a number of mechanisms, including triggering epigenetic modifications and inhibiting transcriptional elongation [Gu et al., 2012; Guang et al., 2010; Kalinava et al., 2017; Mao et al., 2015]. Nuclear RNAi defective (NRDE) factors play essential roles in mediating gene silencing in the nucleus as well as the transgenerational inheritance of RNAi in *C. elegans* (Figure 1) [Ashe et al., 2012; Buckley et al., 2012; Burton et al., 2011; Houri-Ze'evi et al., 2016; Luteijn et al., 2012; Minkina and Hunter, 2017, 2018; Sapetschnig et al., 2015; Shirayama et al., 2012; Spracklin et al., 2017; Weiser et al., 2017]. The germline nuclear Argonaute protein HRDE-1 was identified in a forward genetic screening for factors involved in the RNAi inheritance of a germline expressed *h2b::gfp* transgene targeted by exogenous dsRNAs and in the transgenerational inheritance of piRNA-induced gene silencing [Ashe et al., 2012; Buckley et al., 2012; Shirayama et al., 2012]. HRDE-1 associates with siRNAs to inhibit transcription and direct histone modification at targeted genomic loci. In progeny, HRDE-1 binds to siRNAs complementing the targeted nucleic acids in the parental generation and is also required for the accumulation of small RNAs in later generations [Sapetschnig et al., 2015].

Instead of acting as a *bona fide* carrier to transmit the silencing signal, the nuclear RNAi machinery likely functions in the germline of inheriting progeny to facilitate the memory or re-establishment of RNAi silencing events that occurred in the previous generation. Several lines of evidence cumulatively support this idea. First, the progeny of *nrde(-)* animals exposed to dsRNA still exhibit inherited RNAi silencing at the embryonic stage when animals were fed with dsRNA targeting a soma-expressed transgene [Burton et al., 2011; Xu et al., 2018]. Second, animals that lack HRDE-1 in the RNAi generation but express HRDE-1 in the progeny generation are still able to inherit RNAi silencing [Buckley et al., 2012]. Third, the nuclear RNAi pathway was shown to participate in the maintenance of siRNA expression in the progeny of RNAi-treated animals [Burton et al., 2011; Sapetschnig et al., 2015]. Therefore, the nuclear RNAi machinery is likely not required to directly convey signals from parents to progeny, but it is necessary to perform gene silencing and preserve heritable siRNA expression in progeny.

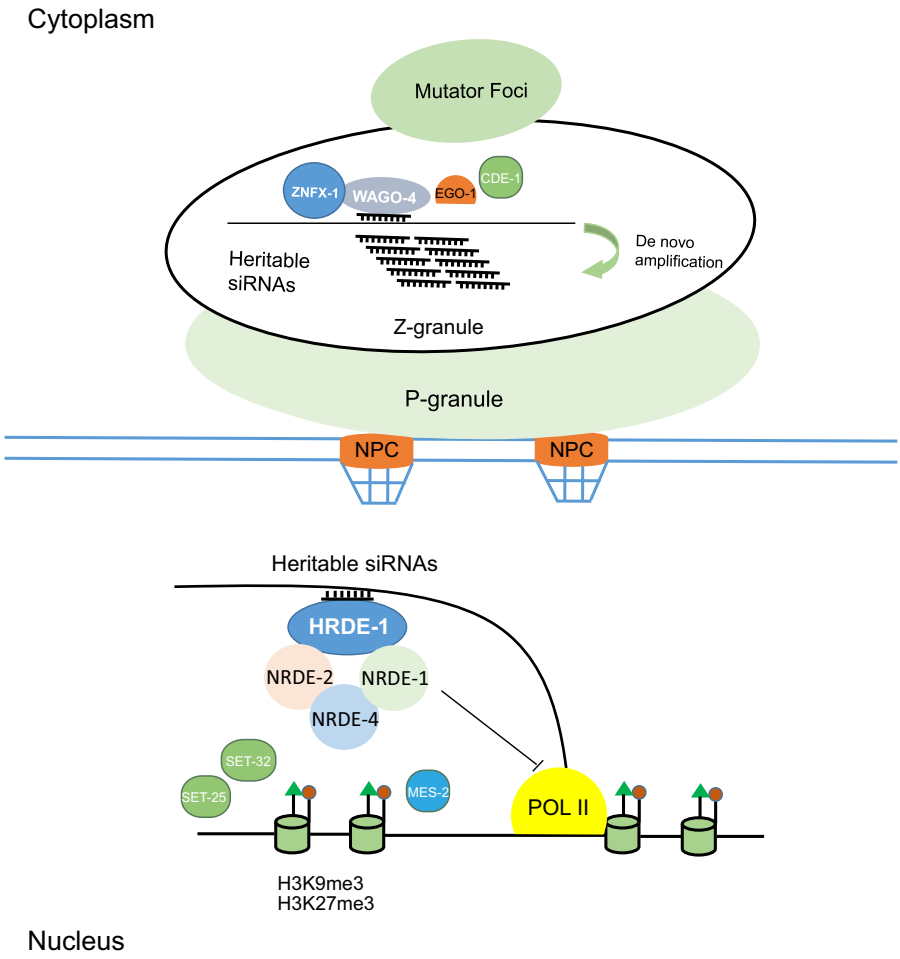
Stable maintenance of silencing requires the generation of additional siRNAs in each generation to avoid the dilution of heritable small RNAs. After the first round of amplification of 22G-RNAs (secondary siRNAs), additional small RNAs (tertiary siRNAs) are synthesised [Sapetschnig et al., 2015]. It was shown that the production of tertiary siRNAs requires a nuclear amplification pathway, which induces the paramutation phenotype and transgenerational RNAi inheritance. Since the majority of Argonaute-associated siRNAs in *C. elegans* are generated by RdRP complex and mutator foci activity upon a mRNA template, which likely takes place in the cytoplasm [Claycomb et al., 2009; Gu et al., 2009; Maniar and Fire, 2011; Phillips et al., 2012], whether and how NRDE factors recruit the cytoplasmic RdRP complex to direct tertiary amplification requires further demonstration.

Chromatin modification factors and the inheritance of RNAi

RNAi-directed chromatin modifications are linked to gene silencing in a variety of organisms. For example, small RNAs initiate H3K9 trimethylation and gene silencing in *Schizosaccharomyces pombe* and *Arabidopsis thaliana* [Moazed, 2009]. In Drosophila, PIWI protein associated with piRNAs and mediated transposon silencing through chromatin modifications [Castel and Martienssen, 2013]. In *C. elegans*, RNAi elicits H3K9me3 and H3K27me3

Figure 1 | A schematic representation of the nuclear and cytoplasmic RNAi machineries in transgenerational inheritance of RNAi

In the cytoplasm, the cytoplasmic Argonaute protein WAGO-4 and the conserved RNA helicase/Zn finger protein ZNFX-1 associate with targeted mRNAs, recruit EGO-1 and synthesise a pool of heritable siRNAs. In the nucleus, the nuclear Argonaute protein HRDE-1 associates with heritable siRNAs, binds to targeted pre-mRNAs and recruits other NRDE factors. The HRDE-1/siRNA/pre-mRNA complex inhibits transcription elongation of RNA polymerase II and induces histone modifications at the targeted loci.



modifications via the Nrde pathway [Gu et al., 2012; Guang et al., 2010; Kalinava et al., 2017; Mao et al., 2015].

Histone modifications are involved in the transgenerational inheritance of RNAi in *C. elegans*. Many chromatin-modifying enzymes and their associated factors, including HPL-2, SET-25 and SET-32, MES-2, MORC-1, K03D10.3, ISW-1 and MRG-1, were identified for the inheritance of RNAi [Ashe et al., 2012; Klosin et al., 2017; Luteijn et al., 2012; Mao et al., 2015; Shirayama et al., 2012; Spracklin et al., 2017; Vastenhouw et al., 2006; Weiser et al., 2017]. In addition, treating *C. elegans* with the histone deacetylase inhibitor trichostatin A can alleviate RNAi silencing in progeny, suggesting that histone acetylation might participate in RNAi inheritance [Grishok et al., 2005].

The precise function of the distinct chromatin modifications in RNAi inheritance is unclear [Rechavi and Lev, 2017]. Two histone methyltransferases, SET-25 and SET-32, are required for RNAi-induced H3K9me3 modification and promote

transgenerational silencing of the GFP reporter gene [Ashe et al., 2015; Luteijn et al., 2012; Sugiyama et al., 2005]. However, H3K9me3 is dispensable for nuclear RNAi-induced transcriptional silencing [Kalinava et al., 2017; Mao et al., 2015]. In a time course experiment, Burton et al. (2011) revealed that siRNAs are detectable before H3K9 trimethylation in progeny and that H3K9me3 marks are more pronounced in progeny than they are in parents, who were directly exposed to the dsRNAs. Therefore, H3K9me3 marks in progeny may be a consequence of re-establishment by inherited siRNAs. It will be very interesting to test whether histone modifications can be inherited independently of small RNAs in *C. elegans*.

A number of results indicated that histone modifications can play a role in inherited siRNA production in progeny. In *A. thaliana* and *S. pombe*, a self-reinforcing feedback loop has been identified in which the RNAi machinery induces H3K9me3 at genomic loci that are targeted by siRNAs and further recruit the RNAi machinery to these loci to generate additional siRNAs and aid the silencing effect [Holoch and Moazed, 2015]. A similar self-reinforcing feedback loop model has been proposed for the function of chromatin modifications in RNAi inheritance in *C. elegans* [Rechavi and Lev, 2017]. MET-2, which is required for H3K9me/H3K9me2, represses heritable RNAi response indirectly, without significantly changing the histone modification at target loci [Lev et al., 2017]. MET-2-dependent H3K9me1/2 correlated with the synthesis of endo-siRNAs, possibly through a reinforcing feedback loop to silence repetitive sequences. The amount of small RNAs generated from endogenous loci, such as repetitive elements, pseudogenes and protein-coding genes, is transgenerationally reduced in *met-2* mutants. In progeny, endogenous siRNAs and exogenous-derived siRNAs compete for Argonaute-related RNAi machineries to further regulate gene expression.

Cytoplasmic RNAi machineries promote RNAi inheritance

Although the nuclear RNAi pathway and chromatin modification factors have been widely accepted to be essential for the transgenerational inheritance of RNAi, recent work has begun to reveal that cytoplasmic RNAi factors also contribute to inheritance (Figure 2).

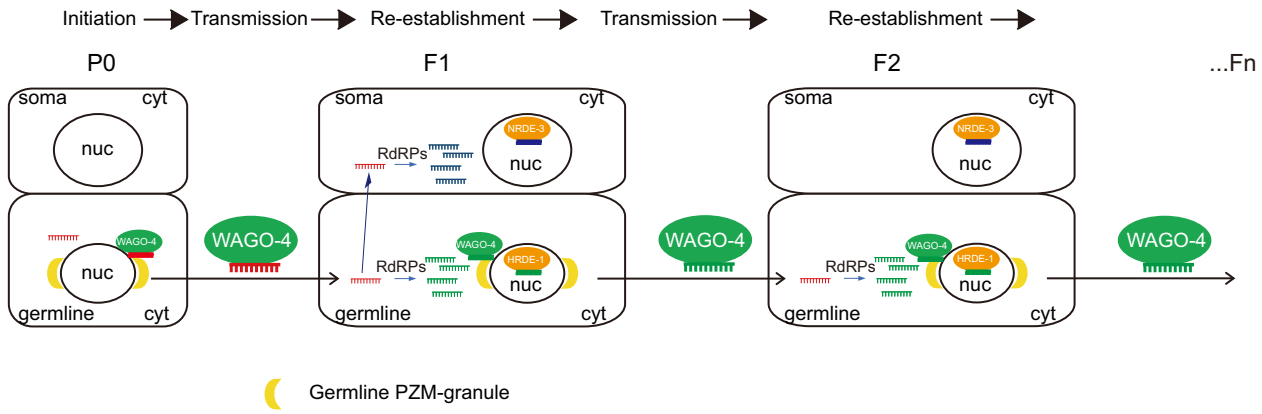
In a forward genetic screening, Spracklin et al. (2017) identified several P-granule-localised proteins, including GLH-1, CDE-1 and HRDE-2. Later, Wan et al. (2018) found that factors involved in P-granule assembly, including DEPS-1, MEG-3/4 and PGL-1, all contribute to RNAi inheritance. Germ granules are self-assembling nonmembrane-bound organelles found in the germ cells of most metazoans [Voronina et al., 2011]. In *C. elegans*, germ granules are referred to as P-granules. P-granules segregate with the germ lineage during embryonic cell divisions [Strome and Wood, 1983]. The mechanism of how these P-granule factors contribute to RNAi inheritance is not fully understood. The concentration of GLH-1/VASA into P-granules and the segregation of these granules with the germline may enable GLH-1/VASA to mediate heritable gene-silencing events. Many RNA processing and regulating factors, including core RNAi factors (such as Dicer, Agos and RdRPs), localise to P-granules [Chen et al., 2016; Claycomb et al., 2009; Conine et al., 2010; Gu et al., 2009; Vasale et al., 2010]. The failure to properly concentrate core RNAi factors in P-granules could be responsible for the defects of RNAi inheritance that were observed in GLH-1 animals. A hypothesis is that P-granule factors could modulate the binding affinity of nuclear RNAi factors to targeted nucleic acids and therefore affect the efficiency of the nuclear RNAi pathway. For example, HRDE-2 was required for RNAi-induced NRDE-2/pre-mRNA association [Spracklin et al., 2017].

Recently, a cytoplasmic Argonaute protein WAGO-4 and a conserved RNA helicase/Zn finger protein ZNFX-1 were identified as essential for transgenerational RNAi inheritance (Figure 1) [Ishidate et al., 2018; Wan et al., 2018; Xu et al., 2018]. WAGO-4 and ZNFX-1 act cooperatively to maintain the expression of heritable small RNAs across generations to promote RNAi inheritance. WAGO-4 associates with heritable siRNAs to target mRNAs and down-regulate targeted mRNAs level. Meanwhile, ZNFX-1 can bind small RNA targeted mRNAs through WAGO-4-dependent manner and recruit RNA-dependent RNA polymerase (RdRP), EGO-1, to synthesise heritable small RNAs.

Interestingly, WAGO-4/ZNFX-1 associates with RNAs and undergoes liquid-liquid phase transitions

Figure 2 | A working model of the transgenerational inheritance of RNAi

The inheritance of RNAi occurs in multiple steps, including initiation, transmission and re-establishment of silencing. WAGO-4 transmits small RNAs from parents to progeny. In progeny, inherited small RNAs targeting soma-expressed genes are translocated to the soma, amplified, and silence somatic gene expression. siRNAs targeting germline-expressed genes are amplified in the germline and silence gene expression in the germline of the progeny. These germline-targeting siRNAs are then transmitted again to the next progeny by WAGO-4, which therefore maintains the RNAi silencing state over multiple generations.



from P-granules and subsequently forms distinct foci between P2 and Z2/Z3 during embryogenesis. In the adult germline, WAGO-4 and ZNFX-1 co-localise in a liquid droplet organelle termed the Z-granule. Z-granules assemble into ordered tridroplet assemblages with P-granules and another germline droplet-like foci termed the Mutator foci [Phillips et al., 2012]. The homolog of ZNFX in yeast is *Hrr1*, which is also involved in RNAi-mediated gene silencing [Motamedi et al., 2004]. It is of great interest to investigate why ZNFX-1/WAGO-4 needs to form distinct foci adjacent to P-granules to promote transgenerational RNAi inheritance. In addition, how P-granules, Z-granules and Mutator foci cooperate with each other to finely regulate gene silencing requires further investigations.

Cytoplasmic RNAi machineries may contribute to RNAi inheritance independently of the nuclear RNAi pathway. RNAi targeting a soma-expressed *sur-5::gfp* transgene silenced *gfp* expression in the parental generation, and this silencing can be inherited by progeny [Burton et al., 2011]. In *hrde-1* mutant animals with a disrupted germline nuclear RNAi pathway, silencing of *sur-5::gfp* is still inherited by F1 progeny. However, in *wago-4* mutants, silencing of *sur-5::gfp* was mitigated in both F1 embryos and larva. WAGO-4 may function in small RNAs transmission from parents to offspring and re-establish the silencing states. Therefore, the cytoplasmic Argonaute protein WAGO-4 and the nuclear Argonaute protein HRDE-1 may act differently or in parallel to promote RNAi inheritance.

dsRNA, the RNAi spreading defective (Rsd) pathway and intergenerational inheritance of RNAi

In addition to siRNAs, dsRNAs have also been reported to be transmitted from parents to progeny in *C. elegans*. By injecting fluorescently labelled dsRNAs, Marre et al. (2016) found that dsRNAs could be imported from the extracellular space into oocytes along with yolk and accumulate within intracellular vesicles in embryos. Subsequent entry into the cytosol of early embryos causes gene silencing in the progeny. Inherited dsRNAs also spread between cells in the progeny to elicit a systemic effect. Consistent with this idea, RNAi spreading defective factors RSD-2 and RSD-6 have been found to promote genome silencing and maintain siRNA populations in progeny [Sakaguchi et al., 2014]. However, whether and how parentally acquired dsRNAs could engage in multigenerational RNAi inheritance requires

further investigation because parentally acquired dsRNAs will be quickly diluted over generations.

RNA uridylation and RNAi inheritance

Poly(U) polymerase-1 (*cde-1*, also known as *pup-1* or *cid-1*) adds short tracts of uracil to the 3′ termini of RNAs in *C. elegans* [Kwak and Wickens, 2007; van Wolfswinkel et al., 2009]. The forward genetic screening of mutants in RNAi inheritance identified *cde-1*, suggesting that uridylation of siRNAs could play important roles in the inheritance processes [Spracklin et al., 2017]. CDE-1 uridylates the endo-siRNAs that bind to germline Argonaute proteins, including CSR-1 and WAGO-4 [van Wolfswinkel et al., 2009]. It was shown that the uridylation might shift the balance of siRNA loading to distinct Argonaute proteins [de Albuquerque et al., 2015; Phillips et al., 2015; van Wolfswinkel et al., 2009]. For example, CDE-1 might destabilise CSR-1 siRNAs but might increase siRNAs binding to WAGO-4. Therefore, CDE-1 promotes RNAi inheritance by modulating WAGO-4-associated 22G-RNAs.

Interestingly, CDE-1 interacts with an RNA-dependent RNA polymerase EGO-1 but not with Argonaute protein CSR-1 [van Wolfswinkel et al., 2009]. EGO-1 is required for the production of 22G-RNAs and likely acts upstream of the loading of 22G-RNAs to Argonaute proteins. Therefore, uridylation happens upstream of small RNA loading onto Argonaute proteins in *C. elegans*. It has been suggested that these 3′ uracil residues may serve as beacons to recruit RdRPs, which amplify small RNA populations and reinforce gene silencing [Tsai et al., 2015]. Alternatively, CDE-1 could function analogously by marking germline mRNAs as templates for RdRP's activity in each generation during RNAi inheritance. Consequently, CDE-1 could maintain small RNA populations at levels sufficient to trigger gene silencing over multiple generations. In the somatic cells of *C. elegans*, the putative poly(U) polymerase MUT-2 may add a uracil to the 3′ end of mRNA fragments produced in response to RNAi [Tsai et al., 2015].

The homolog of CDE-1 in *S. pombe* is Cid12, which is a core factor of the RNA-directed RNA polymerase (RDRC) complex. RDRC consists of *Cid12*, an RNA helicase *Hrr1*, and a RdRP [Motamedi et al., 2004]. RDRC is thought to produce silencing RNAs to perpetuate stable heterochromatic states in *S. pombe* [Martienssen and Moazed, 2015]. It will be very interesting to test whether ZNFX-1, CDE-1 and EGO-1 function as a similar protein complex to mediate the multigenerational inheritance of RNAi in *C. elegans*.

Noticeably, the untemplated addition of 3′-end uracil to 22G-RNAs did not completely disappear in the *cde-1* mutant, indicating the presence of functional redundancy of *cde-1*. *C. elegans*' genome encodes three poly(U) polymerase-1 genes (*cde-1, pup-2* and *pup-3).* It will be interesting to investigate whether PUP-2 and PUP-3 can uridylate 22G-RNAs and mediate RNAi inheritance as well.

Perspectives

Environmental factors, including stress, nutrition or supplementation, can have dramatic impacts on gene expression and development. Although these impacts may not directly alter the genome sequence, they could modulate growth and survival ability and thus affect propagation during the course of evolution [Lim and Brunet, 2013]. However, these evolutionary advantages must be inherited by progeny to accumulate in the population. The transgenerational inheritance of RNAi therefore provides a mechanism to epigenetically transmit environmental information and parental responses to descendants. For example, the small RNA-based antiviral response can be inherited for multiple generations in *C. elegans* [Rechavi et al., 2011]. Interestingly, many mutants defective in RNAi inheritance exhibit a Mrt phenotype in which they gradually lose their progenies along generations [Buckley et al., 2012; Spracklin et al., 2017; Xu et al., 2018].

Many questions remain unanswered regarding the mechanisms and functions of RNAi inheritance. For example, what is the actual silencing signal that is transmitted from parents to their progeny? Why does most transgenerational silencing appear to be restricted to a limited number of generations? Can histone modifications be inherited independently of small RNAs in *C. elegans*? Can inherited silencing information be stably integrated into the genome and permanently shut off gene expression over generations? Due to its short generation time (approximately 3 days) and its ease of culture and genetic manipulation in the laboratory, *C. elegans* is an ideal

model organism for studying the transgenerational inheritance of silencing and testing these hypotheses.

Funding

This work was supported by grants from the Chinese Ministry of Science and Technology (2017YFA0102903), the National Natural Science Foundation of China (Nos. 81501329, 31671346 and 91640110), Anhui Natural Science Foundation (Nos. 1608085MC50 and 1808085QC82) and the Major/Innovative Program of Development Foundation of Hefei Center for Physical Science and Technology (2017FXZY005).

Acknowledgements

We are grateful to the members of the Guang lab for their comments. We are grateful to the International *C. elegans* Gene Knockout Consortium, and the National Bioresource Project for providing the strains. Some strains were provided by the CGC, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440).

Conflict of interest statement

The authors have declared no conflict of interest.

References

Alcazar, R.M., Lin, R. and Fire, A.Z. (2008) Transmission dynamics of heritable silencing induced by double-stranded RNA in *Caenorhabditis elegans*. Genetics **180**, 1275–1288

Ashe, A., Sapetschnig, A., Weick, E.M., Mitchell, J., Bagijn, M.P., Cording, A.C., Doebley, A.L., Goldstein, L.D., Lehrbach, N.J., Le Pen, J., Pintacuda, G., Sakaguchi, A., Sarkies, P., Ahmed, S. and Miska, E.A. (2012). piRNAs can trigger a multigenerational epigenetic memory in the germline of *C. elegans*. Cell **150**, 88–99

Ashe, A., Sarkies, P., Le Pen, J., Tanguy, M., and Miska, E.A. (2015). Antiviral RNA interference against Orsay virus is neither systemic nor transgenerational in *Caenorhabditis elegans*. J. Virol. **89**, 12035–12046

Billi, A.C., Fischer, S.E., and Kim, J.K. (2014). Endogenous RNAi pathways in C. elegans. *WormBook: The Online Review of C elegans Biology*, 1–49

Buckley, B.A., Burkhart, K.B., Gu, S.G., Spracklin, G., Kershner, A., Fritz, H., Kimble, J., Fire, A., and Kennedy, S. (2012). A nuclear Argonaute promotes multigenerational epigenetic inheritance and germline immortality. Nature **489**, 447–451

Burton, N.O., Burkhart, K.B., and Kennedy, S. (2011). Nuclear RNAi maintains heritable gene silencing in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. U. S. A. **108**, 19683–19688

Castel, S.E., and Martienssen, R.A. (2013). RNA interference in the nucleus: roles for small RNAs in transcription, epigenetics and beyond. Nat. Rev. Genet. **14**, 100–112

Chen, J.X., Cipriani, P.G., Mecenas, D., Polanowska, J., Piano, F., Gunsalus, K.C., and Selbach, M. (2016). In vivo interaction proteomics in *Caenorhabditis elegans* embryos provides new insights into P granule dynamics. Mol. Cell. Proteomics **15**, 1642–1657

Claycomb, J.M., Batista, P.J., Pang, K.M., Gu, W., Vasale, J.J., van Wolfswinkel, J.C., Chaves, D.A., Shirayama, M., Mitani, S., Ketting, R.F., Conte, D Jr. and Mello, C.C. (2009). The Argonaute CSR-1 and its 22G-RNA cofactors are required for holocentric chromosome segregation. Cell **139**, 123–134

Conine, C.C., Batista, P.J., Gu, W., Claycomb, J.M., Chaves, D.A., Shirayama, M., and Mello, C.C. (2010). Argonautes ALG-3 and ALG-4 are required for spermatogenesis-specific 26G-RNAs and thermotolerant sperm in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. U. S. A. **107**, 3588–3593

de Albuquerque, B.F., Placentino, M., and Ketting, R.F. (2015). Maternal piRNAs are essential for germline development following de novo establishment of endo-siRNAs in *Caenorhabditis elegans*. Dev. Cell **34**, 448–456

Feng, X., and Guang, S. (2013). Small RNAs, RNAi and the inheritance of gene silencing in *Caenorhabditis elegans*. J. Genet. Genomics **40**, 153–160

Fire, A., Xu, S., Montgomery, M.K., Kostas, S.A., Driver, S.E., and Mello, C.C. (1998). Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. Nature **391**, 806–811

Grishok, A., Sinskey, J.L., and Sharp, P.A. (2005). Transcriptional silencing of a transgene by RNAi in the soma of *C. elegans*. Genes Dev. **19**, 683–696

Grishok, A., Tabara, H., and Mello, C.C. (2000). Genetic requirements for inheritance of RNAi in *C. elegans*. Science **287**, 2494–2497

Gu, S.G., Pak, J., Guang, S., Maniar, J.M., Kennedy, S., and Fire, A. (2012). Amplification of siRNA in *Caenorhabditis elegans* generates a transgenerational sequence-targeted histone H3 lysine 9 methylation footprint. Nat. Genet. **44**, 157–164

Gu, W., Shirayama, M., Conte, D., Jr., Vasale, J., Batista, P.J., Claycomb, J.M., Moresco, J.J., Youngman, E.M., Keys, J., Stoltz, M.J., Chen, C.C., Chaves, D.A., Duan, S., Kasschau, K.D., Fahlgren, N., Yates, J.R. 3rd, Mitani, S., Carrington, J.C. and Mello, C.C. (2009). Distinct argonaute-mediated 22G-RNA pathways direct genome surveillance in the *C. elegans* germline. Mol. Cell **36**, 231–244

Guang, S., Bochner, A.F., Burkhart, K.B., Burton, N., Pavelec, D.M., and Kennedy, S. (2010). Small regulatory RNAs inhibit RNA polymerase II during the elongation phase of transcription. Nature **465**, 1097–1101

Hannon, G.J., Rivas, F.V., Murchison, E.P., and Steitz, J.A. (2006). *The expanding universe of noncoding RNAs*. Cold Spring Harb. Symp. Quant. Biol. **71**, 551–564

Heard, E., and Martienssen, R.A. (2014). Transgenerational epigenetic inheritance: myths and mechanisms. Cell **157**, 95–109

Holoch, D., and Moazed, D. (2015). RNA-mediated epigenetic regulation of gene expression. Nat. Rev. Genet. **16**, 71–84

Houri-Ze'evi, L., Korem, Y., Sheftel, H., Faigenbloom, L., Toker, I.A., Dagan, Y., Awad, L., Degani, L., Alon, U., and Rechavi, O. (2016). A tunable mechanism determines the duration of the transgenerational small RNA inheritance in *C. elegans*. Cell **165**, 88–99

Ishidate, T., Ozturk, A.R., Durning, D.J., Sharma, R., Shen, E.Z., Chen, H., Seth, M., Shirayama, M., and Mello, C.C. (2018). ZNFX-1 functions within perinuclear nuage to balance epigenetic signals. Mol. Cell **70**, 639–649 e636

Kalinava, N., Ni, J.Z., Peterman, K., Chen, E., and Gu, S.G. (2017). Decoupling the downstream effects of germline nuclear RNAi reveals that H3K9me3 is dispensable for heritable RNAi and the maintenance of endogenous siRNA-mediated transcriptional silencing in *Caenorhabditis elegans*. Epigenetics Chromatin **10**, 6

Klosin, A., Casas, E., Hidalgo-Carcedo, C., Vavouri, T., and Lehner, B. (2017). Transgenerational transmission of environmental information in *C. elegans*. Science **356**, 320–323

Kwak, J.E., and Wickens, M. (2007). A family of poly(U) polymerases. RNA **13**, 860–867

Lev, I., Seroussi, U., Gingold, H., Bril, R., Anava, S., and Rechavi, O. (2017). MET-2-dependent H3K9 methylation suppresses transgenerational small RNA inheritance. Curr. Biol. **27**, 1138–1147

Lim, J.P., and Brunet, A. (2013). Bridging the transgenerational gap with epigenetic memory. Trends Genet. **29**, 176–186

Luteijn, M.J., van Bergeijk, P., Kaaij, L.J., Almeida, M.V., Roovers, E.F., Berezikov, E., and Ketting, R.F. (2012). Extremely stable Piwi-induced gene silencing in *Caenorhabditis elegans*. EMBO J. **31**, 3422–3430

Maniar, J.M., and Fire, A.Z. (2011). EGO-1, a C. elegans RdRP, modulates gene expression via production of mRNA-templated short antisense RNAs. Curr. Biol. **21**, 449–459

Mao, H., Zhu, C., Zong, D., Weng, C., Yang, X., Huang, H., Liu, D., Feng, X., and Guang, S. (2015). The Nrde pathway mediates small-RNA-directed histone H3 lysine 27 trimethylation in Caenorhabditis elegans. Curr. Biol. **25**, 2398–2403

Marre, J., Traver, E.C., and Jose, A.M. (2016). Extracellular RNA is transported from one generation to the next in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. U. S. A. **113**, 12496–12501

Martienssen, R., and Moazed, D. (2015). *RNAi and heterochromatin assembly*. Cold Spring Harb. Perspect. Biol. **7**, a019323

Minkina, O., and Hunter, C.P. (2017). Stable heritable germline silencing directs somatic silencing at an endogenous locus. Mol. Cell **65**, 659–670 e655

Minkina, O., and Hunter, C.P. (2018). Intergenerational transmission of gene regulatory information in *Caenorhabditis elegans*. Trends Genet. **34**, 54–64

Miska, E.A., and Ferguson-Smith, A.C. (2016). Transgenerational inheritance: Models and mechanisms of non-DNA sequence-based inheritance. Science **354**, 59–63

Moazed, D. (2009). Small RNAs in transcriptional gene silencing and genome defence. Nature **457**, 413–420

Motamedi, M.R., Verdel, A., Colmenares, S.U., Gerber, S.A., Gygi, S.P., and Moazed, D. (2004). Two RNAi complexes, RITS and RDRC, physically interact and localize to noncoding centromeric RNAs. Cell **119**, 789–802

Phillips, C.M., Brown, K.C., Montgomery, B.E., Ruvkun, G., and Montgomery, T.A. (2015). piRNAs and piRNA-dependent siRNAs protect conserved and essential *C. elegans* genes from misrouting into the RNAi pathway. Dev. Cell **34**, 457–465

Phillips, C.M., Montgomery, T.A., Breen, P.C., and Ruvkun, G. (2012). MUT-16 promotes formation of perinuclear mutator foci required for RNA silencing in the *C. elegans* germline. Genes Dev. **26**, 1433–1444

Rechavi, O., and Lev, I. (2017). Principles of transgenerational small RNA inheritance in *Caenorhabditis elegans*. Curr. Biol. **27**, R720–R730

Rechavi, O., Minevich, G., and Hobert, O. (2011). Transgenerational inheritance of an acquired small RNA-based antiviral response in *C. elegans*. Cell **147**, 1248–1256

Sakaguchi, A., Sarkies, P., Simon, M., Doebley, A.L., Goldstein, L.D., Hedges, A., Ikegami, K., Alvares, S.M., Yang, L., LaRocque, J.R., Hall, J., Miska, E.A. and Ahmed, S. (2014). Caenorhabditis elegans RSD-2 and RSD-6 promote germ cell immortality by maintaining small interfering RNA populations. Proc. Natl. Acad. Sci. U. S. A. **111**, E4323–4331

Sapetschnig, A., Sarkies, P., Lehrbach, N.J., and Miska, E.A. (2015). Tertiary siRNAs mediate paramutation in C. elegans. PLoS Genet. **11**, e1005078

Shirayama, M., Seth, M., Lee, H.C., Gu, W., Ishidate, T., Conte, D., Jr., and Mello, C.C. (2012). piRNAs initiate an epigenetic memory of nonself RNA in the C. elegans germline. Cell **150**, 65–77

Spracklin, G., Fields, B., Wan, G., Vijayendran, D., Wallig, A., Shukla, A., and Kennedy, S. (2017). Identification and characterization of *Caenorhabditis elegans* RNAi inheritance machinery. Genetics 209

Strome, S., and Wood, W.B. (1983). Generation of asymmetry and segregation of germ-line granules in early *C. elegans* embryos. Cell **35**, 15–25

Sugiyama, T., Cam, H., Verdel, A., Moazed, D., and Grewal, S.I. (2005). RNA-dependent RNA polymerase is an essential component of a self-enforcing loop coupling heterochromatin assembly to siRNA production. Proc. Natl. Acad. Sci. U. S. A. **102**, 152–157

Tsai, H.Y., Chen, C.C., Conte, D., Jr., Moresco, J.J., Chaves, D.A., Mitani, S., Yates, J.R., 3rd, Tsai, M.D., and Mello, C.C. (2015). A ribonuclease coordinates siRNA amplification and mRNA cleavage during RNAi. Cell **160**, 407–419

van Wolfswinkel, J.C., Claycomb, J.M., Batista, P.J., Mello, C.C., Berezikov, E., and Ketting, R.F. (2009). CDE-1 affects chromosome segregation through uridylation of CSR-1-bound siRNAs. Cell **139**, 135–148

Vasale, J.J., Gu, W., Thivierge, C., Batista, P.J., Claycomb, J.M., Youngman, E.M., Duchaine, T.F., Mello, C.C., and Conte, D., Jr. (2010). Sequential rounds of RNA-dependent RNA transcription drive endogenous small-RNA biogenesis in the ERGO-1/Argonaute pathway. Proc. Natl. Acad. Sci. U. S. A. **107**, 3582–3587

Vastenhouw, N.L., Brunschwig, K., Okihara, K.L., Muller, F., Tijsterman, M., and Plasterk, R.H. (2006). Gene expression: long-term gene silencing by RNAi. Nature **442**, 882

Voronina, E., Seydoux, G., Sassone-Corsi, P., and Nagamori, I. (2011). RNA granules in germ cells. Cold Spring Harb. Perspect. Biol. **3**

Wan, G., Fields, B.D., Spracklin, G., Shukla, A., Phillips, C.M., and Kennedy, S. (2018). Spatiotemporal regulation of liquid-like condensates in epigenetic inheritance. Nature **557**, 679–683

Weiser, N.E., Yang, D.X., Feng, S., Kalinava, N., Brown, K.C., Khanikar, J., Freeberg, M.A., Snyder, M.J., Csankovszki, G., Chan, R.C., Gu, S.G., Montgomery, T.A., Jacobsen, S.E. and Kim, J.K. (2017). MORC-1 integrates nuclear RNAi and transgenerational chromatin architecture to promote germline immortality. Dev. Cell **41**, 408–423 e407

Xu, F., Feng, X., Chen, X., Weng, C., Yan, Q., Xu, T., Hong, M., and Guang, S. (2018). A cytoplasmic argonaute protein promotes the inheritance of RNAi. Cell Rep. **23**, 2482–2494

Received: 1 June 2018; Revised: 16 July 2018; Accepted: 17 August 2018; Accepted article online: 29 August 2018