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Transgenerational Inheritance: Perpetuating RNAi

Kristen C. Brown[1,2] and Taiowa A. Montgomery[1,*]
[1]Department of Biology, Colorado State University, Fort Collins, CO 80523, USA
[2]Cell and Molecular Biology Program, Colorado State University, Fort Collins, CO 80523, USA
*Correspondence: tai.montgomery@colostate.edu
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Reversible changes in gene expression independent of the genetic code can be transmitted from one generation to the next via poorly understood mechanisms. In worms, a histone-modifying enzyme is necessary to keep small RNA-guided transgenerational gene silencing in check.

RNA interference (RNAi) and related pathways engage ~20–30 nucleotide-long small RNAs to regulate gene expression through base-pairing with target RNAs [1]. Because of their sequence specificity and ability in some instances to be transmitted from parent to progeny, small RNAs are attractive candidates for maintaining a cellular memory of environmentally induced gene silencing from one generation to the next [1,2]. Despite its appeal, there are only a few examples in which small RNAs have been definitively linked to transgenerational inheritance of environmentally triggered changes in gene expression. Some of the most notable examples come from recent studies involving the worm *Caenorhabditis elegans*. Starvation has been shown to trigger the production of small RNAs that can persist for at least three generations in worms [3]. Moreover, silencing of certain viruses and transgenes in *C. elegans* involves a small RNA signal that is epigenetic in nature, as opposed to being encoded in the genome, and is heritably maintained for multiple generations [4–7]. Small RNA-mediated transgenerational gene silencing (transgenerational RNAi) in *C. elegans* is dependent on several chromatin-modifying enzymes [1]. Furthermore, genomic loci corresponding to small RNA targets are often marked with a histone methylation footprint [1], suggesting that small RNAs may impact transgenerational gene silencing by guiding changes to the chromatin landscape of target genes. However, the interplay between small RNAs and chromatin-modifying enzymes and its impact on heritable gene silencing is not well-understood. Adding to the complexity, a new study by Lev *et al.* [8] reported in a recent issue of *Current Biology* identifies a histone-modifying enzyme in *C. elegans* that, instead of being required to maintain gene silencing, has a role in terminating RNAi at each new generation.

Early studies exploring the mechanism of RNAi in worms revealed that in some instances gene silencing is transmitted from the parent receiving RNAi treatment to the progeny of the treated animal [9]. RNAi directed against GFP transgenes, as well as endogenous genes, can persist, albeit somewhat stochastically, for multiple generations before eventually petering out [10]. Lev *et al.* [8] discover that MET-2/SETDB1, an enzyme required for methylation of lysine 9 on histone H3 (H3K9), a chromatin modification associated with silenced regions of the genome [11], functions to diminish inheritance of RNAi from one generation to the next. In the absence of MET-2, a GFP reporter gene targeted by RNAi in a single generation is continually silenced for more than ten generations (Figure 1A).

The mechanisms of RNAi and related pathways differ considerably between worms and mammals. In *C. elegans*, exogenous RNAi is initiated by primary small-interfering RNAs (siRNAs) derived from double-stranded RNA typically administered through feeding or injection. Primary siRNAs, in association with a protein complex that at its core contains the Argonaute RDE-1, guide target mRNAs into a secondary siRNA pathway in which high levels of antisense siRNAs are amplified by RNA-dependent RNA polymerase complexes [1]. The Argonaute HRDE-1 binds to a subset of these secondary siRNAs and intercepts target transcripts in the nucleus, which through an unknown mechanism leads to H3K9 methylation and transcriptional gene silencing [5,6,12] (Figure 1B). HRDE-1, as well as several chromatin factors, including the histone

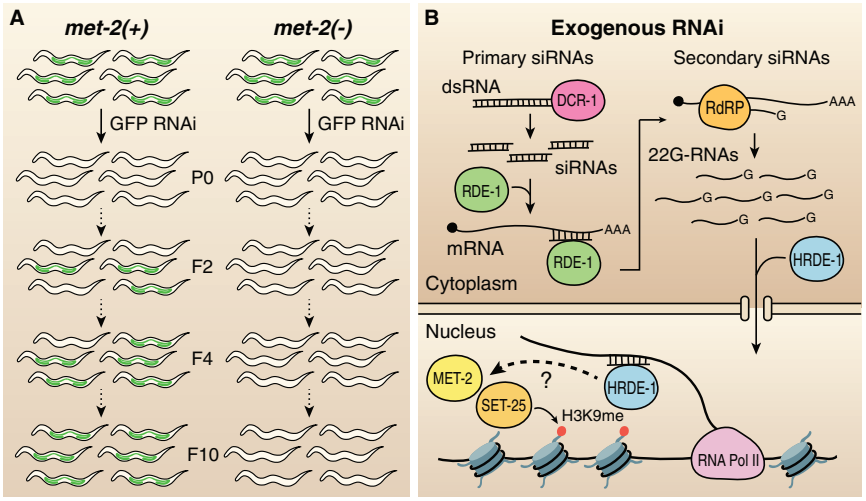

Figure 1. Exogenous RNAi in the *C. elegans* germline.
(A) When subjected to GFP RNAi, worms wild type for *met-2* effectively silence a GFP transgene in the first generation but silencing is gradually lost over multiple generations after removal from RNAi. In *met-2* mutants, GFP silencing persists for more than ten generations following RNAi treatment. (B) The ribonuclease DCR-1/Dicer processes exogenously delivered double-stranded RNA (dsRNA) into ~23-nucleotide duplexes, which are then loaded as single-stranded primary siRNAs into the Argonaute RDE-1. RDE-1 surveils for target mRNAs using the primary siRNAs as sequence-specific guides. Target mRNAs are substrates for a secondary siRNA amplification pathway involving RNA-dependent RNA polymerases (RdRPs). These secondary siRNAs (22G-RNAs) associate with several Argonautes, notably HRDE-1, which effect gene silencing. HRDE-1 delivers siRNAs into the nucleus to intercept nascent transcripts, leading to methylation of histones (H3K9) at target loci by MET-2 and SET-25 through an unknown mechanism.

methyltransferases SET-25 and SET-32, are required for transgenerational RNAi [5,6,12]. In addition to its role in exogenous RNAi, HRDE-1 also acts downstream of endogenous piwi-interacting RNAs (piRNAs) [5,6], a class of small RNAs found in many animals, including humans, that bind Piwi Argonautes to effect silencing of transposons and other foreign genes [1]. In the absence of HRDE-1, the normally immortal germline of *C. elegans* progressively deteriorates until after several generations the animals become sterile [12]. A mortal germline phenotype is also observed in worms bearing a mutation in the *piwi* ortholog *prg-1* [13]. Similar to *hrde-1* and *prg-1*, *met-2* mutants also display germline mortality [14]. In an elegant experiment in which *hrde-1* is knocked out in a *met-2* mutant, Lev *et al.* [8] make the surprising discovery that despite both *hrde-1* and *met-2* mutants displaying germline mortality, the two mutations somehow complement one another to restore immortality to the *C. elegans* germline.

It is unclear what causes germline mortality in either *hrde-1* or *met-2* mutants. Germline mortality in worms lacking PRG-1 and associated piRNAs has been linked to misexpression of repetitive elements [13]. Another recent study implicates small RNAs, MET-2, and several other chromatin factors in silencing of repetitive elements and protecting germ cells from DNA damage and genotoxic stress [15]. It is possible that over generations, misexpression of repetitive elements and mutations caused by transposons gradually accumulate in the absence of the piRNA or HRDE-1 pathway before reaching some threshold that is no longer tolerated. Interestingly, reestablishing endogenous RNAi in worms in which both the piRNA and endogenous RNAi pathways have been disabled causes immediate HRDE-1-dependent sterility. The cause of this sterility likely stems from aberrant silencing of essential genes by HRDE-1 [16,17]. Thus, it is possible that the progressive sterility observed in *hrde-1* and *met-2* mutants is likewise due in part to missilencing of essential genes as the cellular memory of endogenous gene silencing is lost. If HRDE-1 and MET-2 are dependent on each other, not for silencing per se, but rather for targeting the proper genes for silencing, this could potentially explain why mutations in *hrde-1* and *met-2*, while both leading individually to germline mortality, in combination display germline immortality.

In *C. elegans*, transgenerational RNA silencing is accompanied by the production of new siRNAs at each generation, although it is likely that siRNAs are also required to reinitiate RNAi and are maternally transmitted across generations [8,18]. Lev *et al.* [8] show that in the absence of MET-2, siRNAs produced through the exogenous RNAi pathway are elevated over wild type and remain consistently high over multiple generations after removal from RNAi treatment. In contrast, in wild-type worms, siRNAs are gradually lost over each successive generation following RNAi treatment. Progressive changes in endogenous siRNA populations were also observed over several generations in *met-2* mutants. However, it is unclear what affect the observed changes in siRNA levels have on endogenous gene expression.

Despite MET-2 being implicated in H3K9 methylation, Lev *et al.* [8] are unable to identify a direct link between histone modifications and the perpetual RNAi response in *met-2* mutants. Further complicating the story, introducing a mutation in *set-25*, a histone methyltransferase required for H3K9 tri-methylation that is partially redundant with *met-2* [11], reverses the extended transgenerational RNAi phenotype of *met-2* mutants. It is possible that MET-2 has an indirect effect or that its role is related to an unknown molecular function. Despite the mystery behind the mechanism, these observations point to a possible role for histone modifications, either directly or indirectly, in resetting siRNA formation or inheritance at each generation. One explanation consistent with its known role in H3K9 methylation is that MET-2 imparts chromatin modifications that are so effective at inhibiting transcription at HRDE-1 targets that in wild-type worms a minimal pool of mRNA templates is available for siRNA amplification. In this model, insufficient levels of siRNAs are available for transmission to the progeny to reinitiate siRNA amplification and RNAi becomes decreasingly effective over generations. Alternatively, because *met-2*

mutants display enhanced RNAi even in the first generation of treatment, it is possible that MET-2 down-regulates factors that promote heritable RNAi. This model is supported by recent work demonstrating that a feedback response between endogenous and exogenous RNAi pathways controls the duration of heritable RNAi [19].

These new findings raise several important questions concerning the relationship between small RNAs and chromatin modifications that will hopefully steer us towards a clearer understanding of transgenerational inheritance. *C. elegans* has been at the forefront in recent years in identifying roles for small RNAs in transgenerational gene silencing. The extent to which small RNAs affect transgenerational gene silencing in other species is poorly understood. Worms can propagate a small RNA signal at each generation via the activity of RNA-dependent RNA polymerases, a class of enzymes lacking in mammals, and it is unclear if small RNA-based mechanisms of transgenerational silencing exist in humans [2]. However, in flies, which also lack RNA-dependent RNA polymerases, piRNAs are transgenerationally inherited to maintain piRNA production and transposon silencing from one generation to the next [20]. It will be important to identify the mechanisms underlying transgenerational inheritance across different species.

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Human Memory: Brain-State-Dependent Effects of Stimulation

Simon Hanslmayr and Frederic Roux
School of Psychology, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK
Correspondence: s.hanslmayr@bham.ac.uk (S.H.), f.roux@bham.ac.uk (F.R.)
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A new study shows that direct stimulation of memory-relevant brain areas can enhance memory performance, but only when stimulation is applied during brain states associated with poor memory outcome — stimulation during optimal states results in a decrease in memory.

Zaphod Beeblebrox — a character in Douglas Adams, comic sci-fi novel "*The Hitchhiker's Guide to the Galaxy* [1]" — is in a rather confused state when his spaceship lands on planet 'Vogtsphere'. Conveniently, he has at hand a 'thinking