Published in final edited form as:

Nat Genet. ; 44(2): 118–119. doi:10.1038/ng.1088.

The persistence of a silent memory

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Abstract

One of the most striking properties of RNA interference (RNAi) in *Caenorhabditis elegans* is its persistence in the offspring after the triggering double-stranded RNA (dsRNA) has disappeared. A new study reveals that a heterochromatic silencing mark is deposited around the targets of RNAi and transmitted through generations. These results demonstrate that RNAi can induce stable and heritable chromatin modifications in animals.

RNAi is a gene regulatory mechanism that achieves specificity by deriving and utilizing small RNA (sRNA) fragments (20–30 nucleotides) as a complementary guide. An RNA precursor (microRNA, dsRNA or viral RNA) is processed by Dicer nucleases into sRNA, which in complex with Argonaute proteins guide other effectors or act directly on cognate RNA by base-pairing. Initially the mechanism of RNAi was thought to be purely post-transcriptional but soon it was apparent that RNAi could bring about chromatin changes at the targets of sRNA, silencing transcription. The discovery, originally made in the fission yeast *Schizosaccharomyces pombe*¹, elegantly explained the sequence specificity of heterochromatization. However, while mechanistic insights into RNAi-dependent chromatin effects accumulated in plant, fungi and protists, the phenomenon remained elusive in animals. The requirement for RNAi in animal heterochromatin is clear; centromere function is impaired upon disruption of RNAi components², and the control of transposable elements (TE) in the mouse germline, by the Piwi subfamily of the Argonautes leads to the deposition of DNA methylation silencing marks³. However, a direct demonstration of stable transgenerational heterochromatic marking induced by RNAi has been lacking in animals.

This picture is finally changing. Recent work on dsRNA-induced RNAi in *C. elegans* uncovered a nuclear RNAi pathway that downregulates transcription by interfering with RNA Polymerase II transcription and deposits heterochromatic marks in the target gene⁴. On page 157 of this issue, Andrew Fire and colleagues report the molecular mechanism and genetic requirement of this response and demonstrate that dsRNA-induced RNAi establishes heterochromatic marks that persist through several generations⁵. Using chromatin immunoprecipitation followed by high-throughput sequencing they show that dsRNA elicits deposition of the silencing mark Histone 3 Lysine 9 trimethylation (H3K9me3) at specific target loci. Deposition reaches a maximum around the target sequence but also spreads into nearby regions. When directed at an untranscribed region, dsRNA cannot induce H3K9me3, suggesting a required interaction between the trigger sRNA and the transcript.

The authors investigated the mechanism of transgenerational siRNA-induced heterochromatization by analyzing sRNA production. In *C. elegans*, dsRNA is first processed by Dicer into primary small interfering RNA (siRNA). Primary siRNA bind to the Argonaute RDE-1 and guide the cleavage of the endogenous mRNA, which serves as the template for the production of antisense secondary siRNA by RNA-dependent RNA polymerase (RdRP). Other Argonautes bind to the secondary siRNA. Mutation of RDE-1, or a combo mutation of four Argonautes (MAGO) required for secondary siRNA production, abrogated H3K9me3 deposition, indicating that secondary siRNA are necessary to induce chromatin changes. Primary and secondary siRNA appeared before H3K9me3 deposition, but secondary siRNA generation was insufficient; mutant of the nuclear RNAi factor NRDE-2 maintained secondary siRNA accumulation but lost H3K9me3. These results indicate that secondary siRNA, produced independently of heterochromatization, guide H3K9me3 deposition in an NRDE-2-dependent fashion in the nucleus. Strikingly, the H3K9me3 marks persisted in the offspring of the dsRNA-treated animals, with decreasing intensity becoming undetectable after three generations. The decrease mirrored the progressive disappearance of the primary and secondary siRNA.

In a related study, Scott Kenedy and colleagues⁶ also reported the dependence of transgenerational dsRNA silencing on the nuclear RNAi pathway. Interestingly, nuclear RNAi was dispensable for transgenerational silencing of an embryonic gene but necessary for a larval gene. This suggests that nuclear RNAi maintains the silencing signal through embryonic development in the absence of (larval) target mRNA.

These studies set the stage for investigation of RNAi-mediated chromatin modification in animals. The Argonautes implicated in *C. elegans* dsRNA-induced H3K9me3 deposition belong to the WAGO clade, a worm-specific group of Argonautes missing in other species. However, the requirement for secondary siRNA production appears to be conserved. In fission yeast, small primal RNA, derived from degradation of sense-strand RNA, bind Argonaute but only elicit heterochromatin formation with an antisense target and recruitment of RdRP and Dicer activities⁷. Another common requirement is the targeting of a transcribed region. Induction of H3K9me by transfected siRNA in mammalian cells depends on transcription of the targeted promoter⁸, reminiscent of the processing of nascent transcript by RNAi in fission yeast, where once the transcripts are processed, chromatin modification effectors spread into surrounding regions along with the replication fork⁹.

The hunt for the chromatin effectors of RNAi in *C. elegans* now commences and clues may be discerned from other systems; for example, in fission yeast, the RNAi machinery interacts directly with heterochromatin factors¹⁰. Some mechanistic insights are already available. dsRNA-induced RNAi inhibits elongation of nascent transcript⁴; in fission yeast, defects in pre-mRNA processing activate RNA surveillance and result in heterochromatization¹¹.

A recent study by Rechavi *et al.*¹² showed that a virus-induced RNAi also results in siRNA effects that persist through generations, providing an antiviral response in RNAi-defective offspring. The transgenerational silencing signal is likely to be the sRNA itself because RNAi is heritable without the target region. In the absence of reinforcing signals, transgenerational silencing wanes and the H3K9me3 marks eventually disappear. Similar

reinforcing signals may be working on one of the most important natural targets of RNAi: namely heterochromatic TE. In plants, TE expression in the vegetative nucleus of the pollen grain provides an sRNA signal that is transmitted to the sperm nuclei to ensure TE silencing in the zygotic genome¹³. Similarly, the heterochromatic pericentromere is actively transcribed before DNA replication, providing sRNA precursors that ensure heterochromatin inheritance in fission yeast¹⁴. The requirement for nuclear RNAi in transgenerational silencing of non-embryonic genes⁶ also suggests such reinforcing signals at play. In the germline, meiotic silencing also requires RNAi and H3K9me3¹⁵. The unparalleled fate map of cell lineages in *C. elegans* should help reveal how RNAi signals travel through the germline and operate in subsequent generations.

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Figure 1.

A model for transgenerational transcriptional silencing in *C. elegans*. Trigger dsRNA, fed to P₀ worms, is shredded by Dicer nuclease into primary, sense (blue) and antisense (orange), siRNA, and incorporated into the Argonaute RDE-1, which then cleaves the target mRNA. RdRP activities are recruited to produce antisense secondary siRNA (red), which then complex with multiple Argonautes (various shades of green). Among the Argonautes is NRDE-3, which can translocate to the nucleus and inhibit RNA Polymerase II, possibly by localizing to the nascent transcript, and direct H3K9me3 deposition onto nucleosomes (blue ovals). In subsequent generations (F₁, F₂) the H3K9me3 marks persist, but wane as the concentration of primary and secondary siRNA decreases.