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RNA SILENCING

Nuclear RNAi in worms

Many small regulatory RNAs function in nuclei, but the mechanism of nuclear silencing in *Caenorhabditis elegans* has remained enigmatic. Guang *et al*. now identify a nuclear RNA interference (RNAi) mechanism that silences precursor mRNAs (pre-mRNAs) co-transcriptionally and inhibits RNA polymerase II (RNAPII) elongation.

A forward genetic screen for factors required for RNAi in *C. elegans* nuclei previously identified the Argonaute protein NRDE-3, which transports small interfering RNAs (siRNAs) from the cytoplasm to the nucleus. The same screen also identified *nrde-2*, which encodes a conserved and nucleus-localized protein that is required for RNAi — *nrde-2* mutant animals are defective for nuclear RNAi.

Genetic analyses showed that *nrde-2* and *nrde-3* function in the same genetic pathway. NRDE-3-bound siRNAs localize to the nucleus in both wild-type and *nrde-2* mutant animals, suggesting that NRDE-2 functions downstream of NRDE-3. A small amount of NRDE-2 associates with nuclear, but not cytoplasmic, NRDE-3. In addition, RNAi directs NRDE-2 to pre-mRNAs, suggesting that NRDE-2 might be recruited by NRDE-3–siRNA complexes to nascent transcripts that have been targeted by RNAi.

A reverse genetic screen revealed five additional putative nuclear RNAi factors, including the *C. elegans* orthologue of Rpb7, a subunit of RNAPII that functions in siRNA-mediated heterochromatin formation in fission yeast. Although the role of RPB-7 in *C. elegans* nuclear RNAi needs further characterization, it indicates the involvement of RNAPII transcription. Indeed, the association of NRDE-2 and NRDE-3 with unspliced RNAs suggests that nuclear RNAi functions during transcription. In addition, NRDE-2-dependent silencing occurs downstream to sites of RNAi, suggesting that nuclear RNAi is unlikely to occur at transcription initiation but instead during transcription elongation. Chromatin immunoprecipitation analysis revealed a decrease in AMA-1 (the *C. elegans* orthologue of Rpb1, the largest subunit of RNAPII) downstream of the site of RNAi, suggesting that siRNAs might inhibit RNAPII-mediated transcription. This was indeed confirmed by nuclear run-on analysis.

These findings describe a new mechanism for nuclear RNAi in *C. elegans* that is co-transcriptional, and show that small RNAs can regulate RNAPII during the elongation phase of transcription.

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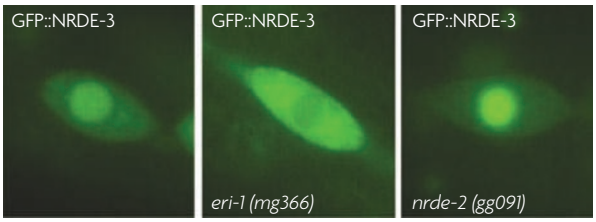

Fluorescent microscopy of a seam cell expressing green fluorescent protein (GFP)–NRDE-3. Arrows indicate nuclei. NRDE-3 binds siRNAs and, in response, localizes to the nucleus similarly in both wild-type (left) and *nrde-2* (*gg091*) mutant cells (right). *eri-1* (*mg366*) mutant animals (middle) fail to express endo-siRNAs and, consequently, NRDE-3 is mislocalized to the cytoplasm. Image is reproduced, with permission, from Guang, S. *et al.* © (2010) Macmillan Publishers Ltd. All rights reserved.

> “a new mechanism for nuclear RNAi”

ORIGINAL RESEARCH PAPER Guang, S. *et al.* Small regulatory RNAs inhibit RNA polymerase II during the elongation phase of transcription. *Nature* 13 Jun 2010 (doi:10.1038/nature09095)
FURTHER READING Hutvagner, G. & Simard, M. J. Argonaute proteins: key players in RNA silencing. *Nature Rev. Mol. Cell Biol.* **9**, 22–32 (2008)