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MILESTONE 4

# Exogenous dsRNA silences genes in *C. elegans*

In 1998, antisense RNA was known to regulate gene expression in cell lines, plants and worms. It was puzzling, however, that in the nematode *Caenorhabditis elegans*, injection of either sense or antisense RNA resulted in transcription interference that could be transmitted to offspring. Fire, Mello and colleagues aimed to uncover some of the mysteries behind what they coined 'RNA interference' (RNAi). They studied the twitching phenotype caused by a reduction in the expression of the gene *unc-22*; complete loss of *unc-22* expression results in more severe muscular defects and impaired motility.

To examine whether single-stranded RNA (ssRNA) or double-stranded RNA (dsRNA) contributed to the twitching effect, a 742-nucleotide ssRNA homologous to *unc-22* was purified and its ability to silence *unc-22* relative to the homologous dsRNA was compared. Whereas the ssRNA produced only incremental effects, co-injection of sense and antisense RNA, rather than consecutive injections, was highly effective in producing a twitching effect in the adult worm. This phenotype was heritable, although progeny were expected to maintain only a few RNA molecules per cell at the 500-cell stage, which is when *unc-22* expression begins.

That gene silencing was likely mediated by dsRNA was further inferred by the fact that injecting the worms with gel-purified dsRNA phenocopied silencing. No effect was observed after injection of control dsRNA that was either not related to or that targeted promoter or intronic regions of *unc-22*. Gene silencing effects could be reproduced by dsRNA, but not ssRNA, that was homologous to three other genes with well-characterized phenotypes. At this point, the authors deduced that dsRNA was involved, that the stoichiometry between the dsRNA and the endogenous target mRNA was not required to be 1:1, and that the response was specific to the targeted mRNA.

To visualize the silencing effect, the authors used *mex-3*, a transcript that is abundant in early embryos and can be easily detected using in situ hybridization. They found that the *mex-3* transcript was not detectable following injection of dsRNA derived from *mex-3*. Surprisingly, injection of purified *mex-3* antisense RNA did not significantly affect *mex-3* transcript levels. Another unexpected finding was that regardless of where in the worm dsRNA was injected, gene silencing was observed in the somatic tissue of the injected worm as well as in its progeny, suggesting the involvement of RNA transport.

> "… dsRNA… response was specific to the targeted mRNA"

Although the mechanism of RNAi remained unclear at the time, this seminal work was the first to show that strong gene silencing can be mediated by dsRNA. It laid the ground-work for a decade of studies that characterized the molecular mechanism underlying the RNAi pathway (MILESTONE 7) and showed that RNAi is a widespread endogenous phenomenon.

Faten Taki, Associate Editor, *Communications Biology*

**MILESTONE STUDY** Fire, A. et al. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* **391**, 806–811 (1998).
**FURTHER READING** Nellen, W. & Lichtenstein, C. What makes an mRNA anti-sense-itive? *Trends Biochem. Sci.* **18**, 419–423 (1993) | Fire, A. et al. Production of antisense RNA leads to effective and specific inhibition of gene expression in *C. elegans* muscle. *Development* **113**, 503–514 (1991).

The 2006 Nobel prize in physiology or medicine is awarded to Andrew Fire and Craig Mello for their discovery of RNA interference — gene silencing by double-stranded RNA.


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