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Paralyzed Worms Add Pieces to the Puzzle of RNA Interference

Using genetically altered strains of the roundworm *C. elegans*, scientists have revealed some of the genetic components responsible for a still-mysterious cellular process called RNA interference (RNAi) in which double-stranded RNA triggers the degradation of a homologous messenger RNA.

The findings show that some of the same genes are involved in RNAi and nonsense-mediated decay, a protective mechanism that may be used by cells to proofread newly created messenger RNA (mRNA) and to prevent the production of defective protein molecules.

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— **Brenda L. Bass**

In an article published in the September 15, 2000, issue of the journal *Science*, Howard Hughes Medical Institute investigator Brenda L. Bass, geneticist Susan Mango and their colleagues at the University of Utah report that three of seven genes of the *smg* gene family are involved in both nonsense-mediated decay and RNAi.

"Nobody knows the purpose of RNAi," said Bass. "All we know is that it seems to be an intrinsic regulatory process, and in these days when we know so much about molecular biology and yet find a new pathway, it's very exciting."

Molecular biologists have used double-stranded RNAi as a tool to degrade mRNA in cells to shut down the effects of specific genes in *C. elegans*, *Drosophila* and many other cell-types. Since nonsense-mediated decay also involves mRNA degradation, Bass and Mango decided to explore whether RNAi requires the *smg* genes, which were known to be involved in nonsense-mediated decay.

To test this possibility, they obtained *C. elegans* strains containing mutations in the various *smg* genes. They next injected these strains and a wild-type strain with double-stranded RNA designed to interfere with production of

myosin, a protein that is critical to muscle development. Thus, the scientists could quantify the effectiveness of RNAi by measuring paralysis in the worms using a crawl assay test of the worms' ability to move across a laboratory dish.

"In wild-type worms, we found that the progeny treated with RNAi were paralyzed from day one and remained paralyzed," said Bass. "So, the RNAi technique completely interfered with their muscle development.

"But when we did the same experiment in worms with mutations in some of the *smg* genes, the RNAi worked very well on day one. But on days two, three and four, the worms recovered until they moved exactly like the wild-type worms." The experiments showed that *smg-2*, *smg-5* and *smg-6* seemed to be required for the persistence of RNAi. According to Bass, the recovery of the worms offers important clues to the relationship between RNAi and nonsense-mediated decay.

"Our data do not say that nonsense-mediated decay is required for RNAi because the worms are paralyzed on day one. But it does seem that some of the *smg* genes are required for persistence of the RNAi effect. This seems to mean that some of the *smg* genes required for nonsense-mediated decay are also playing some sort of direct role in RNAi."

To confirm the connection between the processes, the scientists also performed biochemical experiments in which they monitored the levels of mRNA for the myosin-related gene as the *smg* -mutant animals proceeded through the paralysis induced by RNAi and subsequent recovery.

"We found that, in fact, the mRNA levels for the completely paralyzed worms were very low and as the worms recovered from paralysis, their mRNA levels came up, too," said Bass.

Bass emphasized that the latest findings add only a small piece to a much larger puzzle of nonsense-mediated decay, RNAi and their functions in the cell. "It's difficult to come up with models because we know so little about these processes," she said. "Nonsense-mediated decay is thought to involve some sort of sensing or scanning of the mRNA to make sure it's O.K. in terms of its sequence or perhaps its assembly into a three-dimensional RNA-protein complex. Stop codons in the wrong place are a signal that the mRNA is not OK and should be degraded. It's intriguing to speculate that maybe the interaction of an mRNA with a complementary sequence can feed into this pathway and also provide a signal for degradation.

"Clearly, this is a fascinating unknown frontier in molecular biology, and we have only provided another clue that sets the stage for further experiments," said Bass.