Researchers discover a new role for RNAi

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Organisms employ a fascinating array of strategies to identify and restrain invasive pieces of foreign DNA, such as those introduced by viruses. For example, many viruses produce double-stranded (ds)RNA during their life cycle and the RNA interference (RNAi) mechanism is thought to recognize this structural feature to initiate a silencing response.

Now, UMass Medical School researchers have identified a mechanism related to RNAi that scans for intruders not by recognizing dsRNA or some other aberrant feature of the foreign sequence, but rather by comparing the foreign sequences to a memory of previously expressed native RNA. Once identified, an "epigenetic memory" of the foreign DNA fragments is created and can be passed on from one generation to the next, permanently silencing the gene.

A remarkable feature of this RNAi-related phenomenon (referred to as RNA-induced epigenetic silencing, or RNAe), is that the animal carries a memory of previous gene expression. This memory of active genes serves as an "anti-silencing" signal, which protects native genes from RNAe and under some circumstances appears to adopt foreign genes as self. These findings, described in three studies (including a study by Eric Miska and colleagues of the Gurdon Institute, University of Cambridge and Wellcome Trust, UK) published online yesterday and to appear in the July 6 issue of *Cell*, provide new insights into how identical organisms can have the same DNA sequence but opposite patterns of gene expression and thus dramatically different phenotypes.

"If a worm modulates gene expression by carrying a memory of the genes it expressed in previous generations, perhaps other organisms (including humans) can as well. If so, mechanisms of this type could have an important impact on evolution," said Craig C. Mello, PhD, Howard Hughes Medical Institute Investigator, Blais University Chair in Molecular Medicine and distinguished professor of molecular medicine and cell biology. "The RNAe mechanism could accelerate evolutionary change by increasing heritable phenotypic variation (without the need for DNA mutations). There is growing evidence that many organisms can track and respond epigenetically to gene expression patterns. Our findings provide insight into a whole new level of sophistication in the recognition and memory of gene expression programs."

Dr. Mello and colleagues knew that when a foreign piece of DNA encoding the green fluorescent protein, or GFP, was inserted into the small roundworm C. elegans, some of the worms would silence the newly introduced DNA while others would express the GFP gene. They then explored a role for RNAi in the decision to silence or express GFP. RNAi is a process whereby cells modulate the activity of their genes. In RNAi-related phenomena, Argonaute proteins interact with and use small RNAs as little genetic guides to recognize target nucleic acids through base-pairing interactions.

Based on their findings, Mello and colleagues posit a model comprised of three separate Argonaute systems that work together to scan, identify and silence foreign DNA, while protecting the expression of normal genes. In this system, an Argonaute called PRG-1 (Piwi) bound to piwi-interacting RNA (piRNA) is responsible for scanning molecules of RNA as they leave the nucleus of the cell and determining if they are indigenous to the organism or foreign. If PRG-1 and its piRNA cofactors identify a foreign sequence, it initiates (or activates) the second Argonaute system, known as WAGO, which turns the genetic material off so it can't be expressed.

Once the DNA is identified as foreign and silenced, an epigenetic memory is created that silences the foreign gene from one generation to the next. While the inheritance of this memory requires further exploration, the authors showed that successive generations of C. elegans are unable to express the foreign DNA even if the corresponding piRNA is absent.

"It appears that piRNAs are responsible for the initial scanning and identification of foreign nucleic acids," said Darryl Conte Jr., PhD, research assistant professor of molecular medicine and one of the co-authors on the Cell papers. "Because the foreign DNA in successive generations is being silenced, even in worms that don't have the piRNA, the information necessary for silencing is being passed on epigenetically and independently of the initial scanning done by the piRNA complex in the previous generations."

Originating from clustered regions of the genome, piRNA are diverse and abundant small non-coding RNA molecules in animals, numbering in the millions in mammals. For the most part, piRNAs in worms— and many piRNAs in mammals—lack obvious complementary targets and their function is not clearly understood. It's possible that piRNAs act as a genetic security system, using imperfect base pairing to help identify foreign nucleic acids, said Dr. Conte.

So what prevents piRNAs from recognizing and permanently silencing a gene that the worm identifies as its own? Remarkably, the authors found that such "self" transcripts are somehow protected from entering the WAGO system and that some active genes can actually turn on silent genes. Because the self transcripts are associated with a third Argonaute known as CSR-1, the authors propose that CSR-1 provides an anti-silencing or protective function, which licenses the expression of genes that the worm recognizes as its own.

"This is one of the truly unique findings of these studies," said Conte. "Before, we knew that the RNAi process could be used to regulate genes or to turn them off completely. In this case, what we see is an RNAi mechanism that appears to prevent a gene from being silenced by the piRNA pathway. It works almost as a form of protection that allows the gene to be expressed."

"Taken together, these studies posit a surprisingly complex role for small-RNA systems in epigenetic programming," said Mello. "It shows how piRNAs continuously scan all the genes expressed in the germline, constantly comparing each sequence to a memory of previous gene expression. When foreign genes are recognized and silenced, this new epigenetic knowledge can be passed down to successive generations. On the other hand, occasionally new genes are expressed, apparently stochastically, and this active state too can be passed on as a stable epigenetic memory, thus the organism effectively adopts the foreign gene as self."

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