Argonaute protein slicing, retention of RNA fragments plays role in chemical modification of DNA for gene silencing

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Researchers at Indiana University Bloomington have uncovered previously hidden steps of a gene silencing process used to combat viruses and other would-be genome invaders.

The new findings, published in *Genes & Development*, report the work of a team led by first-author, Dr. Feng Wang in the laboratory of Distinguished Professor of Biology and Molecular and Cellular Biochemistry, Craig Pikaard. The study reveals how a member of an important protein family, ARGONAUTE 4 (AGO4), binds, cuts and retains snippets of ribonucleic acid (RNA) molecules that guide the chemical inactivation of genes with matching sequences.

Gene silencing mediated by RNA molecules is known as RNA interference, or RNAi, and occurs in diverse organisms that include animals, insects, plants, and fungi. There are several different types of RNAi, but all share common features. All begin with RNA polymerase proteins reading the genetic information stored in DNA and copying it into RNA, a process known as transcription.

In all RNAi pathways, double-stranded RNAs (dsRNAs) are synthesized, with the two strands paired like a DNA double-helix. These dsRNAs are cut by Dicer proteins into shorter dsRNAs whose individual strands can range from ~21-35 nucleotides (the individual units of RNA polymers) depending on the species and RNAi pathway. The diced dsRNAs are then loaded into an Argonaute family protein. Only one strand, known as the guide strand, is destined to remain stably associated with the AGO protein.

The other strand, known as the passenger strand, is released and degraded. The AGO protein then uses the guide strand to find RNA targets to which the guide strand can pair, leading to gene silencing by different means. In one scenario, used to inactivate RNAs that encode proteins, the guide programs AGO to slice the target RNA into two fragments or sequester the target RNA away from the protein synthesizing machinery.

In an alternative scenario, pairing of the guide RNA to the target RNA

occurs while the target RNA is still being synthesized and brings about the recruitment of proteins that chemically modify the gene being transcribed. In diverse organisms that include plants and humans, these modifications involve the addition of single-carbon methyl groups to the transcribed DNA, leading to changes in gene organization that prevent further rounds of transcription and RNA synthesis.

In the new study, Wang et al. studied AGO4's role in an RNAi pathway in plants called RNA-directed DNA methylation. The dicer enzyme of this pathway generates both 23 and 24 nucleotide (nt) RNAs, but prior studies had only found 24 nt RNAs associated with AGO4, making the function of the 23 nt RNAs unclear.

A revelation came from analyzing plants engineered to produce AGO4 that is unable to slice target RNAs. In this line, both 23 and 24 nt RNAs were found associated with AGO4. This suggested that 23 nt RNAs normally serve as passenger strands for 24 nt RNAs and are then sliced, a hypothesis supported by recapitulating the reaction in the test tube.

However, the fragments of the sliced 23 nt RNAs were not released, as expected. Instead, they were retained by AGO4, both in the cell and in the test tube. Following up on this observation, Wang and colleagues found that target RNA fragments are also retained by AGO4 after slicing, suggesting that these fragments play a previously unrecognized role in RNA-directed DNA methylation.

Consistent with this idea, the authors found that AGO4's RNA slicing activity is needed to achieve high levels of DNA methylation at target sites throughout the genome. The authors speculate that the retained RNA fragments help tether AGO4-RNA complexes to corresponding DNA sequences to boost the efficiency of DNA methylation.

More information: Feng Wang et al, Enzymatic reactions of AGO4 in

RNA-directed DNA methylation: siRNA duplex loading, passenger strand elimination, target RNA slicing, and sliced target retention, *Genes & Development* (2023). DOI: 10.1101/gad.350240.122