Scientists find the structure of a key 'gene silencer' protein

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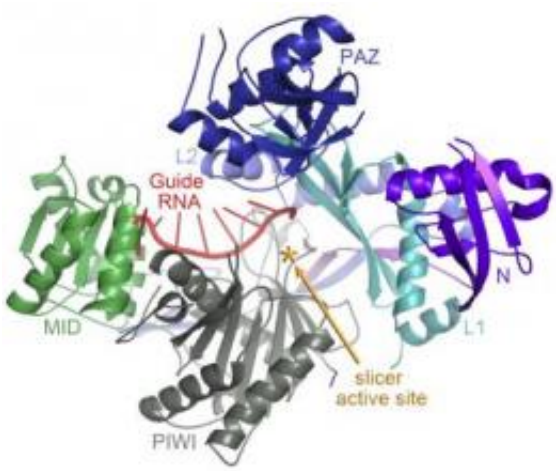

Scripps Research Institute scientists have found the structure of Argonaute2, which can effectively "silence" a gene by intercepting and slicing the gene's RNA transcripts before they are translated into working proteins. Credit: Image courtesy of the MacRae lab, the Scripps Research Institute.

Scientists at The Scripps Research Institute have determined the three-dimensional atomic structure of a human protein that is centrally involved in regulating the activities of cells. Knowing the precise structure of this protein paves the way for scientists to understand a process known as RNA-silencing and to harness it to treat diseases.

"Biologists have known about RNA-silencing for only a decade or so, but it's already clear that there's an enormous untapped potential here for

new therapies," said Ian MacRae, an assistant professor at Scripps Research and senior author of the new report.

The new report, which appeared on April 26, 2012 in the journal *Science*'s advance online publication, Science Express, focuses on Argonaute2. This protein can effectively "silence" a gene by intercepting and slicing the gene's RNA transcripts before they are translated into working proteins.

Interception and Destruction of Messages

When a gene that codes for a protein is active in a cell, its information is transcribed from DNA form into lengths of nucleic acid called messenger RNA (mRNA). If all goes well, these coded mRNA signals make their way to the cell's protein-factories, which use them as templates to synthesize new proteins. RNA-silencing, also called RNA interference (RNAi), is the interception and destruction of these messages—and as such, is a powerful and specific regulator of cell activity, as well as a strong defender against viral genes.

The silencing process requires not only an Argonaute protein but also a small length of guide RNA, known as a short-interfering RNA or microRNA. The guide RNA fits into a slot on Argonaute and serves as a target recognition device. Like a coded strip of VelcroTM, it latches onto a specific mRNA target whose sequence is the chemical mirror image, or "complement," of its own—thus bringing Argonaute into contact with its doomed prey.

Argonaute2 is not the only type of human Argonaute protein, but it seems to be the only one capable of destroying target RNA directly. "If the guide RNA is completely complementary to the target RNA, Argonaute2 will cleave the mRNA, and that will elicit the degradation of the fragments and the loss of the genetic message," said Nicole Schirle,

the graduate student in MacRae's laboratory who was lead author of the paper.

Aimed at disease-causing genes or even a cell's own overactive guide RNAs, RNA-silencing could be a powerful therapeutic weapon. In principle, one needs only to inject target-specific guide RNAs, and these will link up with Argonaute proteins in cells to find and destroy the target RNAs. Scientists have managed to do this successfully with relatively accessible target cells, such as in the eye. But they have found it difficult to develop guide RNAs that can get from the bloodstream into distant tissues and still function.

"You have to modify the guide RNA, in some way to get it through the blood and into cells, but as soon as you start modifying it, you disrupt its ability to interact with Argonaute," said MacRae. Knowing the precise structure of Argonaute should enable researchers to clear this hurdle by designing better guide RNA.

More Points for Manipulation

Previous structural studies have focused mostly on Argonaute proteins from bacteria and other lower organisms, which have key differences from their human counterparts. Schirle was able to produce the comparatively large and complex human Argonaute2 and to manipulate it into forming crystals for X-ray crystallography analysis—a feat that structural biologists have wanted to achieve for much of the past decade. "It was just excellent and diligent crystallography on her part," said MacRae.

The team's analysis of Argonaute2's structure revealed that it has the same basic set of working parts as bacterial Argonaute proteins, except that they are arranged somewhat differently. Also, key parts of Argonaute2 have extra loops and other structures, not seen on bacterial

versions, which may play roles in binding to guide RNA. Finally, Argonaute2 has what appear to be binding sites for additional co-factor proteins that are thought to perform other destructive operations on the target mRNA.

"Basically, this Argonaute protein is more sophisticated than its bacterial cousins; it has more bells and whistles, which give us more points for manipulation. With this structure solved, we no longer need to use the prokaryotic structures to guess at what human Argonaute proteins look like," MacRae said.

He and Schirle and others in the lab now are analyzing the functions of Argonaute2's substructures, as well as looking for ways to design better therapeutic guide RNAs.

"Now with the structural data, we can see what synthetic guide RNAs will work with Argonaute and what won't," MacRae said. "We might even be able to make guide RNAs that can outcompete natural ones."

More information: "The Crystal Structure of Human Argonaute2," *Science*.

Provided by The Scripps Research Institute