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Researchers Reveal New Details about How Argonaute Helps Halt Protein Production

Over the last decade, the study of small RNA molecules has exploded into one of the hottest fields in biology. Small RNAs perform much of the work of regulating how and when genes are switched on and off. For instance, one class of small RNAs, called small interfering RNAs, halts the production of proteins from target genes, and has become an important tool for studying gene function.

Despite the explosion in interest in small RNAs, much of the cellular machinery involved in processing them remains mysterious. Now Howard Hughes Medical Institute investigator Rachel Green and colleagues have discovered new information about how a key class of proteins, called Argonautes, binds to small RNAs and shuts down protein production. Their work, published in the January 10, 2010, issue of the journal *Nature Structural and Molecular Biology*, is helping to reveal one of nature's failsafe mechanisms for ensuring that regulatory RNAs act selectively in halting the production of specific proteins, rather than indiscriminately interfering with the process.

Green, a professor of molecular biology and genetics at the Johns Hopkins University School of Medicine, has spent much of her career studying RNA and how the genetic information it carries is converted by the cell's protein factories – the large complexes of protein and RNA called ribosomes – into proteins. Building on what she and her colleagues have learned about the fundamentals of ribosome function, Green has recently shifted her research to focus on factors outside the ribosome that influence its activity. The ability of small RNAs' to prevent the information carried by messenger RNA molecules from being read by the ribosome was of particular interest – especially since this type of gene regulation is currently being explored as a potential therapy for a variety of diseases.

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- Rachel Green

"It's still unclear exactly how the small RNAs function," says Green. "But it's well known that small RNAs must bind to proteins called Argonautes in order to do their work." She became interested in Argonaute proteins in 2007 when she read an article in the journal *Cell* that suggested Argonautes resemble the eIF4E protein. That comparison piqued Green's curiosity because eIF4E helps translate the genetic message carried by messenger RNA (mRNA) molecules. Messenger RNA molecules are the genetic templates for proteins. In constructing proteins, the mRNA template is transcribed from DNA genes and transported to the ribosomes.

To do its job, eIF4E grabs onto the end of mRNA and helps guide it to the ribosome. The binding of eIF4E to the cap of an mRNA molecule "is the universal signal that says, 'I'm a genetic message, you should translate me,'" says Green. The paper Green read suggested that Argonautes might also latch onto the cap of the mRNA molecule – the same section to which eIF4E binds.

Argonautes' potential interaction with mRNA deserved a closer look, Green said, because if Argonaute proteins also bound to the mRNA cap, they would interfere with eIF4E – and thereby interfere with protein production.

While the experiments reported in the *Cell* paper were intriguing, it seemed there was more to be learned, so Green and Sergej Djuranovic, a post-doctoral fellow in her lab, decided to investigate the issue themselves. First, Djuranovic compared the genetic sequence of a small section of the Argonaute proteins, called the MID domain, to DNA sequences deposited in Genbank, the genetic sequence database at the National Institutes of Health. Immediately, Djuranovic saw that the Argonaute MID domain did not resemble any part of eIF4E. Instead, Djuranovic found that the MID domain was related another class of bacterial proteins involved in binding nucleotides– the building blocks of DNA and RNA.

"Sergej is a protein guy, and he said, 'Let's make these MID domains and see what they do,'" recalls Green.

Green and her team quickly discovered that the MID domain does in fact bind the cap of mRNA molecules. The team made MID domains from Argonaute proteins from a variety of organisms, including humans, worms, and fruit flies, and Argonautes from a certain class bound tightly to mRNA caps – interestingly, Argonaute proteins that are not involved in translational repression did not appear to bind as well. The scientists later confirmed that

the complete Argonaute proteins bound the mRNA cap just as the isolated MID domain did.

When they tested whether other molecules – specifically nucleotides – might get in the way of Argonaute binding to an mRNA cap, the scientists were surprised at what they found. Although they had suspected that the nucleotides might physically block Argonaute’s cap-binding site, instead they found that adding nucleotides to the mix actually enhanced Argonaute’s ability to bind to an mRNA cap. “The Argonaute proteins didn’t behave as they should if they had just one binding site,” says Green. “That told us something interesting was going on.”

After a long series of experiments conducted on isolated proteins and fruit fly cells in culture dishes, Green and her team solved the puzzle. When an Argonaute protein grabs onto a small RNA molecule, the Argonaute changes its shape slightly. This shape change helps Argonaute get a better grip on a second molecule – messenger RNA. This two-step process matches the small RNA – which has a specific genetic sequence – to its corresponding mRNA. In that way, after binding a small RNA, an Argonaute protein “knows” which mRNA to molecule to seek out and help destroy. “As soon as an Argonaute binds a small RNA, that’s when it starts looking at genetic messages, or mRNA,” says Green.

Djuranovic’s database searches found that Argonaute proteins strongly resemble certain proteins found in bacteria, so these observations suggested that the Argonaute proteins have a deep evolutionary history. “What’s super exciting is that the core of this Argonaute protein is ancient,” says Green. “From that simple core structure evolved this beautiful function of using small RNAs to regulate various important functions throughout all of biology.”