Caught in the act: 3-D structure of an RNA-modifying protein determined in action

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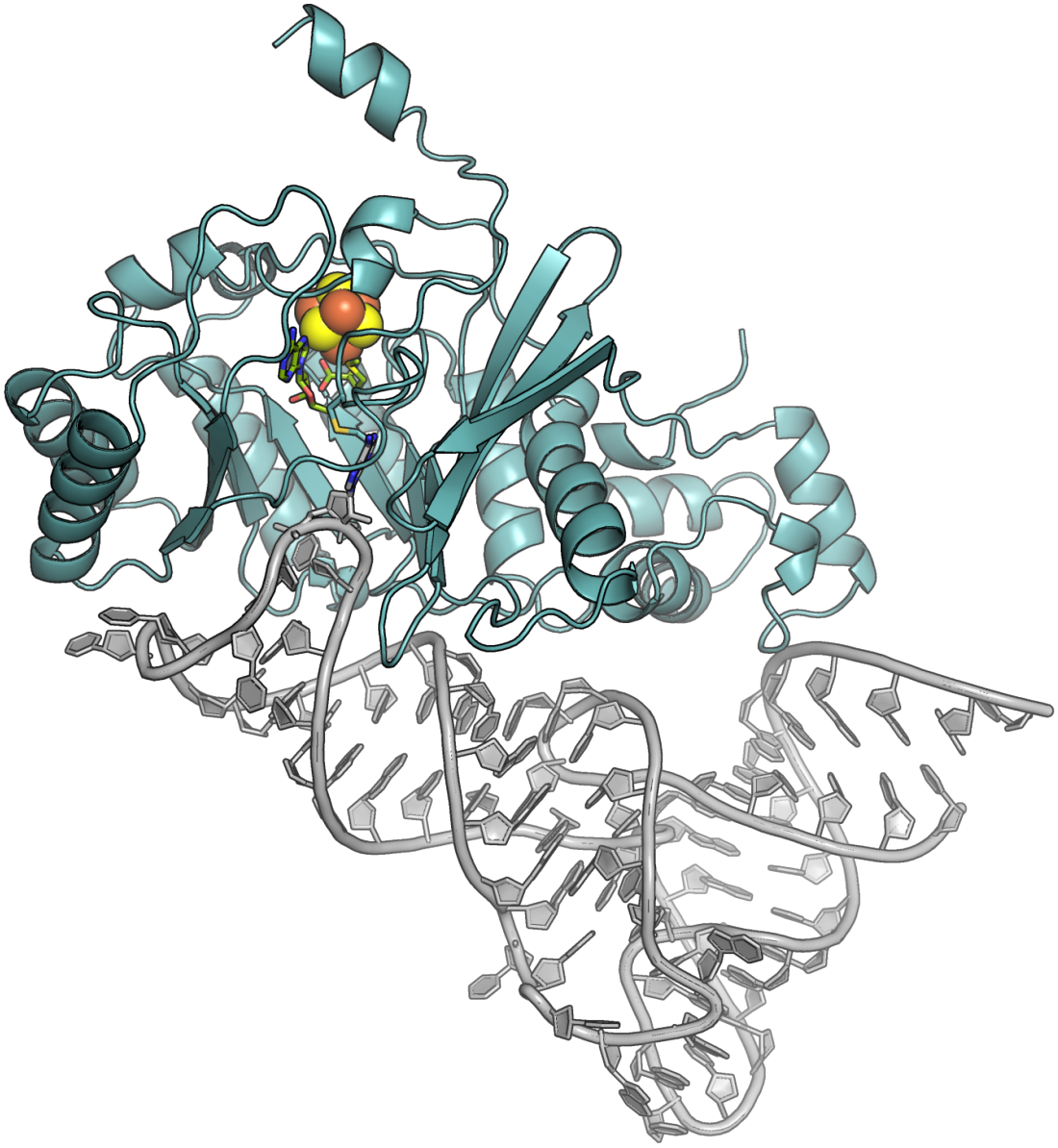

The structure of a bacterial RNA-binding protein has been determined in the act of modifying a molecule of RNA -- an achievement that provides researchers with a unique view of the protein's function in action and could lead to clues that would help in the fight against the development of antibiotic-resistant infections. A paper describing the findings by a team of Penn State University researchers is published in the journal *Science*. This image is a ribbon diagram showing the structure of the RNA-modifying protein RlmN. The RlmN (blue ribbon) is trapped in the middle of its reaction while it is bound to transfer RNA (shown in grey, stick format). Iron and sulfur atoms are shown as orange and yellow spheres. Selected amino acids, cofactors, and nucleobases are shown in stick format and are colored by atom type. Credit: Penn State University

The structure of a bacterial RNA-binding protein has been determined in the act of modifying a molecule of RNA—an achievement that provides researchers with a unique view of the protein's function in action and could lead to clues that would help in the fight against the development of antibiotic-resistant infections. A paper describing the findings by a team of Penn State University researchers is published in the current issue of the journal *Science*.

"We have captured and characterized the structure of the protein, RlmN, at a key intermediate stage in its interaction with the RNA molecule," said Squire J. Booker, professor of chemistry and of biochemistry and molecular biology at Penn State University, an investigator of the Howard Hughes Medical Institute, and one of the leaders of the Penn State research team. "RlmN makes chemical modifications to RNA molecules that function to ensure that the bacteria can accurately synthesize new proteins. By having a picture of this intermediate step, we can learn a lot mechanistically about the protein's function."

The research team determined the three-dimensional structure of the RlmN protein from the bacterium, *Escherichia coli*. They took advantage

of a mutation in the RlmN protein that results in a stable chemical bond between the protein and the RNA molecules it modifies. This approach allowed the researchers to capture and determine the structure of the protein during this normally transient event.

"RlmN is one of only two proteins that are known to make chemical modifications to at least two different kinds of RNA molecules. In fact, RlmN technically modifies seven RNAs—it can modify a site on ribosomal RNA and six different transfer RNAs," said Booker. "RlmN is very closely related, both evolutionarily and functionally, to a protein that functions to confer antibiotic resistance in bacteria, Cfr. Because RlmN and Cfr function by very similar mechanisms, we can use our structure of RlmN bound to one of its substrates to understand better how Cfr confers antibiotic resistance. Ultimately, the structure may help design drug molecules to combat antibiotic resistance."

Until recently, the sole activity of the RlmN protein was believed to be modifications of ribosomal RNA—the large RNA molecules that catalyze protein synthesis in the cell. Surprisingly, the researchers trapped a version of RlmN in the middle of its reaction cycle bound to another, much smaller type of RNA molecule. Their discovery occurred while they were attempting to capture the RlmN protein while it was modifying ribosomal RNA in *E. coli* cells. The researchers determined the structure of this protein/RNA complex and showed that the RlmN protein was bound to a molecule of transfer RNA. Transfer RNA also is involved in building proteins in the cell.

The researchers were able to refine the resolution of their structure of the RlmN protein bound to the transfer RNA by producing the complex in test tubes with purified protein and synthesized molecules of transfer RNA. Both structures show that, unlike other proteins that make chemical modifications to transfer RNA, RlmN interacts with the entire length of the transfer RNA molecule. An extended RNA interface may

be linked to the ability of RlmN to modify a larger RNA substrate like ribosomal RNA in addition to the smaller transfer RNA molecules.

"Not many RNA modifying proteins can target different types of RNA, and RlmN is very different from the only other known protein that modifies both ribosomal and transfer RNAs. The difference is that RlmN does not recognize the sequence of the RNA molecules," said Amie Boal, assistant professor of chemistry and of biochemistry and molecular biology at Penn State and another leader of the research team. "RlmN instead recognizes the three-dimensional structure of its target RNA. In fact, the protein actually remodels the RNA dramatically to make it fit within the rigid structure of the protein's active site. This distinctive approach to substrate recognition is probably the key to how the protein targets two very different types of RNA molecules."

More information: E. L. Schwalm et al, Crystallographic capture of a radical S-adenosylmethionine enzyme in the act of modifying tRNA, *Science* (2016). DOI: 10.1126/science.aad5367

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