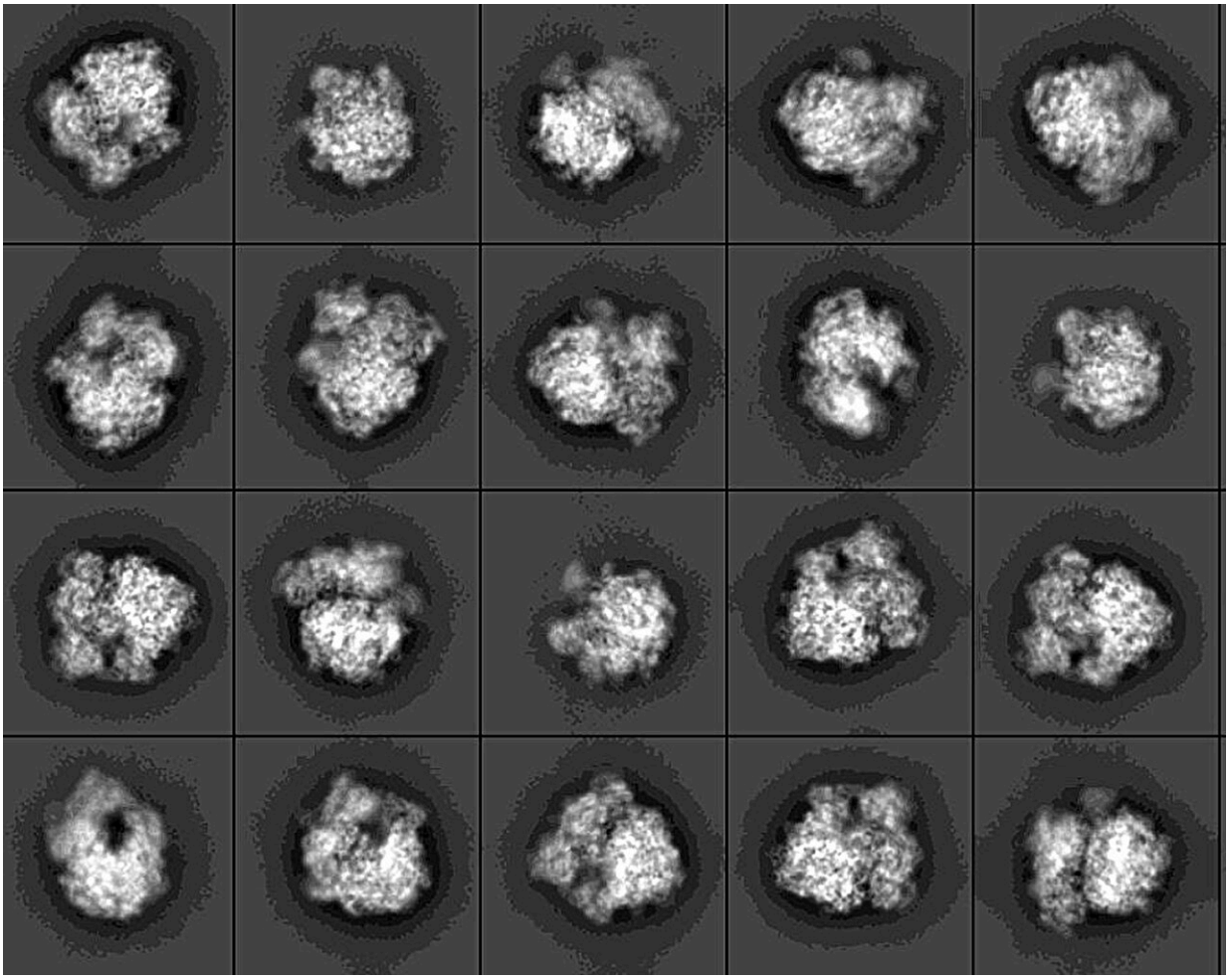
Images of enzyme in action reveal secrets of antibiotic-resistant bacteria

August 14 2023, by Carol Clark



A series of images captured through cryoelectron microscopy shows how a bacterial enzyme modifies a ribosome. Credit: Pacific Northwest Center for Cryo-EM

Bacteria draw from an arsenal of weapons to combat the drugs intended to kill them. Among the most prevalent of these weapons are ribosome-modifying enzymes. These enzymes are growing increasingly common, appearing worldwide in clinical samples in a range of drug-resistant bacteria.

Now scientists have captured the first images of one important class of these enzymes in action. The images show how the enzymes latch onto a particular site on the bacterial ribosome and squeeze it like a pair of tweezers to extract an RNA nucleotide and alter it. The research, led by scientists at Emory University, have been published in the *Proceedings of the National Academy of Sciences* (*PNAS*).

The advanced technique of cryoelectron microscopy made the ultra-high-resolution, three-dimensional snapshots possible.

"Seeing is believing," says Christine Dunham, Emory professor of chemistry and co-corresponding author of the paper. "The minute you see biological structures interacting in real life at the atomic level it's like solving a jigsaw puzzle. You see how everything fits together and you get a clearer idea of how things work."

The insights may lead to the design of new antibiotic therapies to inhibit the drug-resistance activities of RNA methyltransferase enzymes. These enzymes transfer a small hydrocarbon known as a methyl group from one molecule to another, a process known as methylation.

"Methylation is one of the smallest chemical modifications in biology," says Graeme Conn, professor of biochemistry in Emory's School of Medicine and co-corresponding author of the paper. "But this tiny modification can fundamentally change biology. In this case, it confers resistance that allows bacteria to evade an entire class of antibiotics."

Both Conn and Dunham are also members of the Emory Antibiotic Resistance Center.

First author of the paper is Pooja Srinivas, who did the work as a Ph.D. candidate in Emory's graduate program in molecular and systems pharmacology. She has since graduated and is now a postdoctoral fellow at the University of Washington.

Understanding the ribosome

Dunham is a leading expert on the ribosome—an elaborate structure that operates like a factory within a cell to manufacture proteins. Proteins are the machines that make cells run while nucleic acids such as DNA and RNA store the blueprints for life. The ribosome is made mostly of RNA, which does not just store information but can also act as an enzyme, catalyzing chemical reactions.

One goal of Dunham's lab is to find ways to manipulate bacterial ribosomes to make them more susceptible to antimicrobials. If an antimicrobial successfully inactivates bacterial ribosomes, that shuts down the manufacturing of proteins essential for bacterial growth and survival.

The idea is to exploit differences in human cellular ribosomes and bacterial ribosomes, so that only the bacteria is targeted by an antimicrobial drug.

Antimicrobials, however, need to get past bacterial defenses.

"It's like a molecular arms race," Dunham explains. Bacteria keep evolving new weapons as a defense against drugs, while scientists evolve new strategies to disarm bacteria.

Enzymes that modify the ribosome

Conn is a leading expert in the bacterial defense weapons known as ribosomal RNA methyltransferase enzymes. This family of enzymes was originally discovered in soil bacteria. They are now increasingly found in bacterial infections in people and animals, making these infections harder to treat.

"They keep turning up more and more often in clinical samples of some nasty bacterial pathogens in different parts of the world," Conn says.

The enzymes can drive deadly drug-resistance in pathogens such as E. coli, Salmonella, Klebsiella pneumoniae, Pseudomonas aeruginosa and Enterobacteriaceae. The enzymes add a methyl group at a specific site on the bacterial ribosome. That addition blocks the ability of a class of antibiotics known as aminoglycosides to bind and cause their antibacterial action.

For the *PNAS* paper, the researchers focused on a culprit within this family of enzymes known as ribosomal RNA methyltransferase C, or RmtC.

A complicated enzyme

For decades, researchers have relied on a technique known as X-ray crystallography to reveal the atomic details of how molecular machines work when the molecules are arranged in a crystal.

In 2015, for example, Dunham's lab obtained precise pictures through X-ray crystallography of how an enzyme known as HigB rips up RNA to inhibit growth of the bacteria. By restraining the growth of the bacteria that makes it, HigB establishes a dormant "persister cell" state that

makes the bacteria tolerant to antibiotics.

The secrets of how the RmtC enzyme interacts with the ribosome, however, eluded X-ray crystallography.

"RmtC is much more complicated," Dunham explains. "It's an interesting enzyme from a basic science perspective because it looks so different from others."

A resolution revolution

Recent advances in cryoelectron microscopy opened the door to zooming in on the complex mechanisms of RmtC.

Cryoelectron microscopy does not require crystallization to reveal the structures of molecules and how they interact. Instead, liquid samples are frozen rapidly to form a glassy matrix. The glassy matrix retains the three-dimensional structure of molecules and protects them from deterioration by the intense electron beam.

Meisam Nosrati, a former postdoctoral fellow in the Conn lab and a co-author of the PNAS paper, prepared samples of RmtC interacting with part of an E. coli ribosome. He tapped the expertise of co-author Lindsay Comstock, a chemist at Wake Forest University who developed a technique to trap and stabilize the enzyme in the needed position.

Nosrati then froze the samples on a tiny grid and sent them to the Pacific Northwest Center for Cryo-EM for imaging.

As a graduate student in the Dunham lab, Srinivas then analyzed and interpreted the microscopy dataset. She used computer algorithms to stitch together thousands of individual images. The result turned the images into a flipbook that revealed the complicated structure of RmtC

in action.

"The enzyme latches on like a pincer to the ribosome," Dunham explains. "It tightens its grip until it squeezes out a nucleotide from the interior of an RNA helix. It then chemically modifies that nucleotide."

The enzyme is exquisitely specific about where it binds to the ribosome, a huge macromolecule made up of 50 different proteins and 6,000 different RNA nucleotides.

The researchers used biochemistry techniques to validate that what they observed matched previous findings for how RmtC makes bacteria resistant to aminoglycoside antimicrobials that target the ribosome.

Strategies for new therapies

The researchers are now trying to develop new ways to counter the effects of RmtC and related enzymes based on the new information.

"Knowledge of the shape of the enzyme as its performs its chemical reaction gives us new targets to inhibit its effects," Conn says. "For instance, we could target the pincer action of the enzyme to try to prevent it from squeezing and binding to the ribosome. We now know that the enzyme forms a pocket on its surface where a small molecule might sit to block this action."

More information: Pooja Srinivas et al, 30S subunit recognition and G1405 modification by the aminoglycoside-resistance 16S ribosomal RNA methyltransferase RmtC, *Proceedings of the National Academy of Sciences* (2023). DOI: 10.1073/pnas.2304128120

Provided by Emory University