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Researchers Make Gains in Understanding Antibiotic Resistance

Howard Hughes Medical Institute researchers chiseling away at the problem of antibiotic resistance now have a detailed explanation of how the drugs' main cellular target in bacteria evolves to become resistant to some of these medications. The findings are already leading to new experimental antibiotics that are being engineered to circumvent resistance, which is a major worldwide health problem.

Led by Thomas A. Steitz, a Howard Hughes Medical Institute investigator at Yale University, and Peter B. Moore, a professor of chemistry at Yale, the research team published its findings in the April 22, 2005, issue of the journal *Cell*.

Steitz and his colleagues studied the structural basis of bacterial resistance to a group of antibiotics that, while chemically quite different, all jam the activity of the protein-making factory in bacteria in much the same way. They studied the MLS_BK antibiotics, an acronym for a group of antibiotics which include macrolides, lincosamides, streptogramin B and ketolides. MLS_BK antibiotics work by binding to the RNA, near the peptidyltransferase center, of the large subunit of the ribosome. The ribosome is the molecular machine responsible for translating the genetic information on messenger RNA into the long strings of amino acids called polypeptides that are used to build the cell's enzymatic machinery.

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- Thomas A. Steitz

"These antibiotics are clinically very important, and resistance to such antibiotics is a major health problem," said Steitz. "It is becoming critical to understand the precise structural basis of resistance and even more important to do something about it." Steitz cited, for example, recent statistics published in the journal *Nature*, stating that hospitals in the United States see

some two million cases of antibiotic-resistant infections each year; 90,000 patients die annually from such infections.

In their experiments, Steitz and his colleagues used x-ray crystallography to do high-resolution structural analyses of the large ribosomal subunits bound to a number of the MLS_BK antibiotics. In this analytical technique, intense beams of x-rays are directed through crystals of proteins. The underlying atomic structure of the proteins is deduced by analyzing the pattern of diffraction of the x-rays.

Steitz's group used ribosomal subunits from the primitive archaeobacterium *Haloarcula marismortui* (*Hma*), which is found in the Dead Sea. They chose *Hma* ribosomes for their studies because they crystallize well enough to yield high-resolution structural data, but these ribosomes, like those from eukaryotes, are resistant to most MLS_BK antibiotics.

The researchers analyzed the structure of erythromycin - among the most widely prescribed macrolide antibiotics - bound to a mutated version of the *Hma* ribosome that corresponds to a form found in pathogenic bacteria. Their studies revealed details of erythromycin binding to the mutant form of the *Hma* ribosome that do not agree with similar analyses by other researchers, according to Steitz. The studies by Steitz's group yielded new information about the basic chemical principles that underlie binding of the antibiotic to the ribosome, as well as new data about how that mutation confers drug resistance.

Steitz and his colleagues also analyzed the structure of five other clinically important antibiotics - azithromycin, telithromycin, clindamycin, and virginiamycin M and virginiamycin S - bound to the large mutated ribosomal subunit. Steitz said these studies provided new details about the nature of drug resistance involving these antibiotics. Furthermore, the studies of the two forms of virginiamycin offer an explanation for how the two forms of that antibiotic work synergistically to kill bacteria.

Finally, the researchers used x-ray crystallography to explore at high resolution the structural basis of a particular ribosomal mutation dubbed L22 that confers resistance to macrolides such as erythromycin. In a seeming paradox, said Steitz, this mutation confers resistance, even though the antibiotic still binds to the mutant ribosome.

The new structural data indicate that the L22 mutation increases the size of a "tunnel" in the ribosome, through which the growing peptide chain moves during synthesis. This tunnel is normally blocked by macrolide antibiotics. In the mutant form, the tunnel widens, which may explain why macrolide antibiotics are no longer effective.

According to Steitz, insights about the ribosomal origins of antibiotic resistance are already being applied to the development of new antibiotics.

One company leading the way is Rib-X Pharmaceuticals, which was founded by Steitz and colleagues at Yale.

“About half of current antibiotics target the ribosome, and most of them target the large subunit,” he said. “So, such advances have the potential for significant clinical impact. The general strategy of Rib-X to overcome resistance is to create new hybrid antibiotics that possess the ability to bind to interact simultaneously with different, nearby sites on the ribosome represented by different classes of antibiotics,” he said.

“The idea is to take a bit of one antibiotic and tie it to another. So, if resistance arises due to a mutation in one site, there is still another binding site that can be targeted. It's like multiple drug therapies for HIV, in which the drugs attack several sites at once. And if the virus mutates to avoid the effects of one drug, it still gets hit by another. However, in the case of these antibiotics, the binding sites are linked in one molecule. It's like a multi-drug treatment, but in one compound,” he said.