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Enzyme's Structure Yields Blueprint for Antibiotic Resistance

Starting with the structure of a common bacterial enzyme, a team of investigators from the Howard Hughes Medical Institute at The Rockefeller University has unlocked the structural secrets at the heart of some of life's most important biological processes.

Using x-ray crystallography, HHMI investigator Stephen K. Burley and his colleagues, working with Alex Vassilev, Yasutaka Makino and Yoshihiro Nakatani of the National Institute of Child Health and Human Development, have generated the three-dimensional molecular structure of a common enzyme, known as aminoglycoside 3-N-acetyltransferase, from the bacterium *Serratia marcescens*. The research is reported in the August 21, 1998, issue of the journal *Cell*.

"Blocking the enzyme, aminoglycoside 3-N-acetyltransferase (right), could prevent bacteria from inactivating antibiotics, says HHMI investigator Stephen K. Burley."

According to Burley, this work has far-reaching repercussions because the bacterial enzyme's structure is shared by a superfamily of at least 150 enzymes present in every kingdom of life. Across the members of this family, says Burley, minor differences on the surfaces of the molecules generate small changes in structure, allowing them to perform a variety of biological functions.

The HHMI team determined that the bacterial enzyme's structure resembles "a cupped right hand wrapped around a cylinder." Named in part for its function, the enzyme promotes the transfer of an acetyl group from a donor molecule to a recipient molecule. "The three-dimensional structure shows us how the enzyme positions both the donor and the recipient so that the transfer can occur," Burley says.

Using a technique called "threading," Burley's team, which included Rockefeller scientists Eva Wolf and Andrej Sali, used the structure of the single enzyme they discerned to examine other members of the family of

enzymes without actually having to complete x-ray crystallography on each complex. "Essentially, we thread the amino acid sequence of the unknown structures through the structure of the protein we do know and ask, 'Is it a good fit?'" Burley says. Using this technique, the researchers determined that all 150 highly conserved enzymes share the same basic structure.

Three closely related member of the acetyltransferase superfamily exist in a growing number of bacteria and they can affect humans in a potentially life-threatening manner. These enzymes transfer an acetyl group to compounds known as aminoglycosides-widely prescribed antibiotics that include gentamicin, tobramycin and netilmicin.

"The transfer inactivates these antibiotics, making them ineffective. This is an example of emerging antibiotic resistance at work," says Burley. "Now that we know the structure of the enzymes, it may become possible to design a drug that can be given along with the antibiotic. A drug that blocks the enzymes could prevent the antibiotic from being inactivated." There is precedence for such a drug, Burley says, pointing to augmentin, which blocks the part of the enzyme that inactivates the antibiotic penicillin.

Other important acetyltransferases abound. One enzyme with the same fundamental structure is responsible for the final chemical step in synthesizing a neurotransmitter important in the control of mood, depression and anxiety. In another incarnation, a structurally similar enzyme acts as a switch that frees DNA to become active and begin expressing messenger RNA. Such widespread biological activity gives the discovery importance for a wide range of investigations.

Discerning the common structure of such a large family of enzymes also provides an important clue to understand how proteins accomplish life's functions. Burley explains that although there are some 100,000 human genes, they produce only about 2,000 basic protein structures. Slightly different surfaces give the proteins their unique chemical properties and abilities, but in many cases, their basic structures are shared. "We now know one more of the fundamental structures," Burley says.