UCSD Biochemists Discover Bacteria's Achilles' Heel

Research May Aid Design of Novel Antibiotics

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Researchers at the University of California, San Diego have determined what factors turn on protein production in bacteria, a finding that provides new targets for the development of antibiotics.

In the study, published in the April 7 issue of the journal *Molecular Cell*, researchers Sean Studer and Simpson Joseph in UCSD's Department of Chemistry and Biochemistry report how the messenger RNA instructions to make a protein are unfolded in a bacterial cell, so that they can be read by the cell's protein-making machinery. Since unfolding the instructions is an essential step in the making of a protein, the researchers say that drugs designed to interfere with this step would make ideal antibiotics.

"With antibiotic-resistant strains of bacteria on the rise, there is a crisis in the management and treatment of these infections throughout the world," said Simpson Joseph, a professor of chemistry and biochemistry who led the study. "Our results will provide insights for developing novel antibiotics that target the messenger RNA unfolding process in disease-causing bacteria."

Messenger RNA (mRNA) feeds through a ribosome-protein factory in a cell-like a tape through a teletype machine. There the RNA instructions are read and a protein is assembled, one amino acid building block at a time. However, mRNA is usually folded up like origami. Until now, scientists did not understand how the mRNA in bacteria was unfolded so it could be read by the ribosome.

"It's been known for about 10 years that in humans and other complex organisms there is a specialized unwinding mechanism that requires a number of different proteins working in cooperation," explained Sean Studer, a chemistry and biochemistry graduate student who conducted the research. "But the process is not the same in bacteria, and while there is a great deal of research on protein synthesis in bacteria, the unfolding step is one aspect that has been overlooked."

In order to determine what factors were needed for the unraveling process to occur, Joseph and Struder designed a test that used fluorescence to signal when an mRNA strand unwound. They made mRNA with different fluorescent molecules attached to either end. When the mRNA was twisted around on itself, the two fluorescent molecules were in close proximity and could exchange energy, resulting in a change in the color of the fluorescence detected. Unfolding of the mRNA separated the fluorescent molecules and prevented the color change.

The fluorescence test showed that the mRNA did not unfold when in the presence of ribosomes alone. Joseph and Studer discovered that unfolding required a protein called initiation factor 2 as well as initiator tRNA- a molecule that carries the first amino acid of the protein described by the mRNA instructions. In addition, the mRNA must contain a small region, the Shine-Dalgarno sequence, that allows it to bind to the ribosome. The researchers say that their study reveals vulnerabilities in bacterial protein production that can be exploited to design new antibiotics. "Initiation factor 2, initiator tRNA and the Shine-Dalgarno sequence are great targets because they are essential to the unfolding process and they are conserved in bacteria," said Joseph. "Since

mRNA unfolding in human cells is a different, more complex process that doesn't require these factors, drugs that inactivate them should not harm human cells."

The researchers say that the fluorescence test they developed could be a valuable tool to quickly identify compounds that block the mRNA unfolding in bacteria and have the potential to be used as antibiotics.

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