

NOVEMBER 14, 2008

The Path to Self-Destruction: How Antibiotics Kill Bacteria

Antibiotics revolutionized medicine without anyone knowing how the drugs actually work. Since the first antibiotics reached the pharmacy in the 1940s, researchers discovered that they target various pieces of machinery in bacterial cells, disrupting the bacteria's ability to build new proteins, DNA, or cell wall. But these effects alone do not cause death, and a complete explanation of what actually kills bacteria after they are exposed to antibiotics has eluded scientists.

Opening up that antibiotic black box has become increasingly urgent, says James J. Collins, a Howard Hughes Medical Institute investigator at Boston University. "The emergence of antibiotic resistant bacteria is calling for the need to develop new insights into how antibiotics work and to develop more effective antibiotics," he says.

"Prior to our work of a year ago it was thought that antibiotics killed primarily, if not solely, through the drug-target interactions."

— James J. Collins

Collins and his colleagues have made a significant step toward that goal. They traced the sequence of metabolic events that kill *Escherichia coli* bacteria when they are treated with the antibiotic gentamicin, and found that two cellular stress response systems were activated in cells killed by antibiotics, regardless of the drug's initial action. The discovery, described in the November 14, 2008, issue of *Cell*, could point toward new ways of combining existing antibiotics to make therapies more powerful.

Gentamicin is an aminoglycoside—one of three classes of antibiotics that kill bacteria rather than simply stop their growth. These broad-spectrum drugs are common in prescription eye drops, ointments, and injections that treat a variety of infections.

Researchers have known since the 1960s that aminoglycosides bind to the bacteria's protein-building factory - the ribosome - and cause it to assemble proteins incorrectly.

But Collins and his team reported in the journal *Cell* last year on the discovery of an additional mechanism that contributes to the drugs' lethal effects. The group found that all bactericidal antibiotics, regardless of their initial targets inside bacteria, caused *E. coli* to produce unstable chemicals called hydroxyl radicals. These compounds react with proteins, DNA, and lipids inside cells, causing widespread damage and rapid death for the bacteria. "Prior to our work of a year ago it was thought that antibiotics killed primarily, if not solely, through the drug-target interactions," Collins said.

Collins and his colleagues followed up that work with a series of experiments designed to find out what happens between aminoglycosides binding to ribosomes and induction of the newly discovered hydroxyl radical cell death pathway. Collins, doctoral student Michael Kohanski, and their collaborators at Boston University used microarray analysis to measure the activity of *E. coli* genes and pinpoint those that became more or less active when the bacteria were exposed to gentamicin. From among the 650 genes that responded, the researchers identified six networks of interacting genes, based on comparison to an *E. coli* gene network map previously constructed in the Collins lab.

They also screened nearly 4,000 mutant strains of *E. coli*, each of which was missing a single gene, looking to see if gentamicin treatment caused any changes in growth rate. A nonlethal dose of gentamicin made 303 of the strains grow faster than the average gentamicin-treated strain, while the drug slowed growth in 11 of the mutant strains.

With the results of these two experiments, the researchers were able to identify three major processes implicated in gentamicin-induced cell death: protein transport, a stress response triggered by abnormal proteins in the cell membrane, and a metabolic stress response.

The team hypothesized that some of the abnormal proteins produced by gentamicin-compromised ribosomes must be transported to the cell membrane. When the membrane stress response system detects these abnormal proteins, it prepares to remove and degrade them. The membrane stress response system is also linked to a metabolic stress response system which, if over-activated, ultimately leads to production of the deadly hydroxyl radicals.

The researchers gained more support for this model by studying eight different mutant *E. coli* strains. In each of these, one component of the membrane-protein quality control or stress response systems had been disabled. They found that mutants that allowed more mistranslated proteins to enter the membrane initiated the stress response sooner and died more quickly. Those with disabled stress response systems survived gentamicin treatment several hours longer than controls. Indeed, knocking out the stress response systems also made the bacteria more tolerant of ampicillin and norfloxacin, which target different bacterial machinery than gentamicin. These results confirm that, regardless of the initial drug target, the same systems put bacteria on the path to producing the deadly radicals, Collins

says.

Collins now wants to solve the pathways that lead to stress response activation for the other drugs. "We'll move on to other classes of antibiotics to work out the black box, the specific triggers," he says. He plans to use those results "to gain insight into how to convert bacteriostatic drugs [those that halt bacterial growth] into bactericidal drugs [those that kill], to identify targets that one might hit to make such a conversion." Most antibiotics target proteins that are essential to cell survival, Collins says. But they may also kill cells by targeting proteins—like those in the membrane quality-control system—that are not themselves essential but that nevertheless speed antibiotic-induced cell death.