BIOCHEMISTRY

Hijacking a Cell's Chemical Paths to Make New Antibiotics

The push is on to find new versions of old wonder drugs, as ever more strains of bacteria develop resistance to conventional antibiotics. The trouble is, many antibiotics have a complex molecular architecture that makes them extremely difficult to synthesize, let alone tinker with afterward. For these molecules, medical researchers have largely had to accept what nature gives them: compounds made by antibiotic-producing bacteria and fungi. In this issue of *Science*, however, a group of biochemists describes a way to hijack the antibiotic-producing chemical pathways of bacteria, exploiting them to produce a wide variety of new compounds.

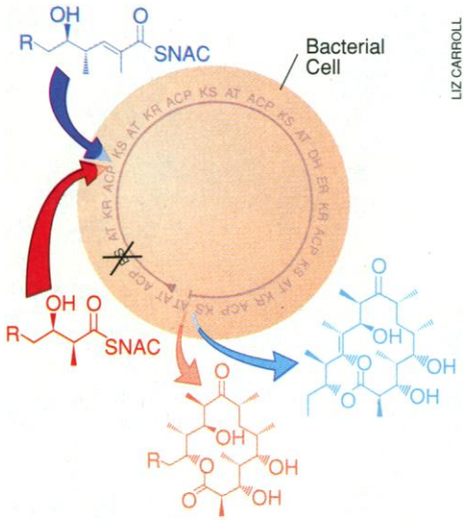
To construct their natural products, these bacteria rely on an assembly line of about 30 enzymes in which each enzyme hands off its product to the next. Now Chaitan Khosla, a chemical engineer at Stanford University in Palo Alto, California, and his colleagues have managed to interrupt this assembly line partway through, replace the natural intermediate compound with an altered one, and restart the process. As they report on page 367, the new intermediate is taken up by the enzymes and incorporated into the growing chemical structure. By simply changing intermediates, the researchers can get the bugs to construct a variety of antibiotic analogs, many of them well suited to being manipulated further with standard medicinal chemistry techniques.

"It's a nice piece of work," says Leonard Katz, an antibiotics researcher at Abbott Laboratories in North Chicago, Illinois. "It opens the door to making a bunch of new molecules quickly"—molecules that can then be evaluated as potential new antibiotics, antifungals, or anticancer compounds. Katz is doubtful, however, that the technique could be scaled up for industrial production, as it requires researchers to add synthetic starting molecules that may be time-consuming and expensive to make.

Still, just finding candidate compounds is half the challenge in discovering new drugs. To date, one of the richest sources of promising molecules has proven to be a family of complex natural compounds called polyketides, most of which are made by bacteria and fungi with their involved, assembly-line process for use as defensive chemical weapons. Of the thousands of polyketides discovered thus far, hundreds have already been tapped as pharmaceuticals. That success rate has sent researchers looking for ways to generate new polyketide variants.

By tinkering with the genes for the enzymes that are the "workers" in the assembly-line process, scientists can alter the end product. But "it's still a relatively cumbersome process to make modifications by altering an organism's genes," says Khosla. What's more, the variety of novel polyketides this strategy can produce is limited, because the organisms must assemble their compounds from molecular building blocks at hand in the cell.

Chemists, of course, can produce a far richer array of building blocks. So Khosla and his colleagues—Stanford postdoc John Jacobsen, Richard Hutchinson of the University of Wisconsin, Madison, and David Cane of Brown University in Providence, Rhode Island—decided to give polyketide-producing bacteria some new building blocks to work with. The researchers started with *Streptomyces coelicolor*, an organism that is easy to manipulate genetically. They had previously engineered the bacterium to express the entire series of 28 enzymes needed to make the common polyketide antibiotic erythromycin.



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Break and switch. By interrupting an enzymatic assembly line and substituting new molecules for the natural intermediate, biochemists can alter the final product.

Using conventional genetic-engineering techniques, they disabled the third enzyme in the series. That prevents it and enzymes 4 through 6 from working together to take up pairs of a natural, molecular building block—a small, three-carbon chain compound called propionic acid—and from stitching them together into a single six-carbon chain. Enzyme 7 normally takes up this chain and passes it on down the assembly process. But without this six-carbon chain, "the other enzymes don't get what they need to do their thing, and the whole system comes to a grinding halt," says Khosla.

To reboot the system, the researchers synthesized molecules slightly different from the six-carbon chain that enzyme 7 normally uses as its feedstock and then substituted them for the original. When eight-carbon building blocks were added to the *S. coelicolor*'s fermentation bath, for example, the stand-ins were taken up by enzyme 7 and passed along until a final, new polyketide, possessing two extra carbons, emerged at the end of the assembly line. The result shows that "these enzymes seem to be very tolerant to using new substrates, and that's good news for making novel natural products," says Khosla.

Enzyme number 7 and its downstream brethren even accepted more radical changes, such as one building block with a six-carbon ring linked to a six-carbon chain, transforming these into a variety of new polyketides. A final processing step at the end to add sugar groups that are normally present on erythromycin turned these products into active antibiotics, says Cane. What's more, the activity of these compounds can be fine-tuned, because they have chemical structures not found in natural polyketides, such as double bonds between adjacent carbon atoms. Medicinal chemists are adept at modifying such structures.

Despite this promise, "the technique still has a long way to go before it's ready to produce molecules for market," says Katz. He points out that even though *S. coelicolor* turns out the new products as efficiently as it normally produces erythromycin, other organisms used in commercial manufacture of the drug have been engineered to produce quantities 1000 times larger. And unless the novel polyketides can be produced at this level, it's doubtful the process would be commercially viable, says Katz. He also points out that while erythromycin building blocks are all produced for free as natural metabolites in bacterial cells, the building blocks for the analogs must be specially synthesized, which can be both time-consuming and expensive.

Cane, however, sees no reason why the biochemistry of commercial organisms couldn't be hijacked as well, turning them into factories of new compounds. The need for custom-made building blocks isn't a major obstacle either, Khosla says. He notes that the building blocks he and his colleagues tested took only three or four steps to make, which he calls "within the range of what synthetic chemists are comfortable producing." A few extra steps could be a small price to pay if the strategy leads to new weapons against antibiotic-resistant bacteria.

–Robert F. Service