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## A Ringing Endorsement for New TB Drug Target

By knocking out a single ring-like component of a molecule on the surface of *Mycobacterium tuberculosis*, researchers have created a mutant strain of the deadly bacterium that fails to establish a lethal chronic infection in mice. The gene knockout abolishes the bacterium's ability to form the braided, serpentine colonies that signify virulence.

The research provides a new target for the development of drugs that may drastically shorten treatment for tuberculosis (TB) infections that afflict 32 percent of the world's population and kill two million people annually, says William R. Jacobs, Jr., a Howard Hughes Medical Institute investigator at Albert Einstein College of Medicine.

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In an article in the April 2000 issue of *Molecular Cell*, Jacobs and colleagues Michael S. Glickman and Jeffery S. Cox report that they created a mutant TB strain by developing and successfully employing a powerful new technique to knock out genes in TB bacteria. Their "phage-mediated knockout" technique reduces the time required to knock out genes in TB bacteria. Such timesaving could greatly increase the pace of research on the disease, say the scientists.

"A principal reason that tuberculosis continues to be a major world health problem predicted to kill more people this year than ever before is its remarkable ability to persist in the body," said Jacobs. "This persistence requires six months of continuous chemotherapy for successful treatment of the disease. So, our aim has been to improve treatment by studying the factors that lead to this persistence."

Jacobs and his colleagues began their experiments by using a transposon (a DNA sequence that can insert itself anywhere in the genome) to create a huge array of mutants of *Mycobacterium bovis*. *M. bovis* is closely related to *M.*

*tuberculosis*, but is safer to manipulate. When the scientists examined the 3,500 colonies of mutants that they had created, they found two strains that failed to produce the ropelike colony structures called "cording" that are characteristic of virulent strains of the bacterium. Cording was first described by Robert Koch in 1882 when he discovered *M. tuberculosis*.

When Jacobs and his colleagues looked for the gene that had been inactivated by the transposon in one of the two mutants, they found that the disrupted gene was one that had already been identified by the TB gene-sequencing project. The disrupted gene coded for an enzyme that modifies mycolic acids extremely long-chain lipid molecules that cover the surfaces of both *M. bovis* and *M. tuberculosis*. Comparing the sequence of the disrupted gene to sequences in gene databases revealed that it was likely a cyclopropane synthetase, an enzyme that catalyzes the formation of a three-membered carbon ring structure on the end of the mycolic acid, alpha mycolate. Thus, Jacobs and his colleagues called the gene *pcaA*, for "proximal cyclopropanation of alpha mycolates."

To see whether *pcaA* played the same role in *M. tuberculosis* that it did in *M. bovis*, the researchers inactivated the *pcaA* gene in *M. tuberculosis* using the phage-mediated knockout technique. "This technique is a very powerful method that I believe will revolutionize the way that we make knockouts in TB," said Jacobs. "It has enabled us to reduce the time required to produce knockouts from six months down to a few weeks."

They used a tuberculosis-infecting phage that was engineered to inject gene segments that lacked the *pcaA* gene into the TB bacteria. Knocking out the *pcaA* gene in *M. tuberculosis* disrupted the cording in those bacterial colonies, as it did in *M. bovis*. Biochemical analysis revealed that the *M. tuberculosis pcaA*-knockout also had an altered alpha mycolate that lacked the cyclopropane ring.

The most dramatic difference between wild-type tuberculosis and the knockout bacterium, however, became evident when the scientists infected mice with the two organisms.

"Amazingly, while the wild-type strain kills the mice, the knockout strain lacking only a single ring structure on a mycolic acid does not," said Jacobs. "The knockout strain grows just as well as the wild-type during the first three weeks, but then is unable to persist normally within the animal."

Thus, said Jacobs, targeting *pcaA* and other genes that code for molecules that are essential for virulence could have a profound impact on treatment.

"Development of drugs to target this cyclopropane synthetase could give us a far more powerful ability to kill this persistent organism, possibly reducing treatment time from six months to two weeks," he said. "While we don't know if this enzyme is *the* ideal drug target, we have certainly found the first one," he added.