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Preventing Biofilms Could Help Fight TB

HHMI researchers have identified a gene that enables mycobacteria—the cause of tuberculosis and leprosy—to form biofilms. Bacterial biofilms help mycobacteria resist treatment. But researchers found that when mycobacteria closely related to the TB and leprosy pathogens lack one key protein, mature biofilms fail to form. Interrupting the gene that produces this protein, known as GroEL1, could help treat or prevent these dread diseases.

To decipher the protein's role in biofilm construction, Graham F. Hatfull, a Howard Hughes Medical Institute (HHMI) professor at the University of Pittsburgh, collaborated with HHMI investigator William R. Jacobs, Jr., at Albert Einstein College of Medicine. They discovered that GroEL1 oversees the production of a particular set of fatty acids called mycolic acids, which are necessary for biofilm growth.

Hatfull is one of 20 scientists nationwide who received \$1 million each from HHMI to help bring the excitement of research into the science classroom. He works with undergraduates and Pittsburgh area high school students to identify bacteriophages, common viruses that infect bacteria. A bacteriophage infecting *Mycobacterium smegmatis*, a non-pathogenic cousin of *Mycobacterium tuberculosis*, helped launch the study that Hatfull and Jacobs report in the December 2, 2005 issue of the journal *Cell*.

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- **Graham F. Hatfull**

"We've defined one of the first genes and mechanisms through which mycobacteria form biofilms," said Hatfull. "Understanding biofilms is important because bacteria in biofilms are tolerant to most antibiotics, and

this tolerance is a major problem in controlling TB infections,” he explained.

TB infects one in three people worldwide and kills thousands each day in third-world countries. Infections can also persist undetected for a lifetime. “Biofilms could play an important role in how TB itself can hunker down and protect itself from drugs and immune effector killing mechanisms. Perhaps TB hangs out in a biofilm somewhere in the body,” suggested Jacobs. “If so, an understanding of biofilm formation will provide novel ways to develop more effective drugs to fight TB and other mycobacterial infections.”

Biofilms are associated with antibiotic resistance in some bacterial infections, including *Streptococcus* and *Pseudomonas* respiratory infections. For bacteria, biofilms are an important survival tool—consisting of communal layers of bacterial cells attached to a liquid or solid surface.

They stubbornly persist, hindering treatment with antibiotics. Physically, a biofilm forms a stronger, less accessible structure than a loosely clumped colony of bacteria. And metabolically, biofilm cells are believed to function in an energy-saving mode.

“Bacteria have evolved many ways to go into this hunker-down phase, and one way is biofilms,” said Jacobs. “We’re not sure yet for TB, but this work provides the unprecedented expected new role for a long-known protein involved in phage production, namely GroEL1.”

Jacobs' and Hatfull's current study began with the unexpected observation by Hatfull's postdoctoral fellow, Anil Ohja, that a virus-infected strain of *Mycobacterium smegmatis* could not form proper biofilms. The virus, the mycobacteriophage Bxb1—named the Bronx Bomber by Jacobs after he isolated it from dirt in his own backyard in the Bronx, New York—integrates its DNA into the middle of the mycobacterium's *GroEL1* gene. This integration disrupts production of the GroEL1 protein, which belongs to a class of proteins known as chaperones that help shape and guide other proteins within the cell.

While another chaperone protein, GroEL2, is essential for mycobacterial growth, the strain missing GroEL1 managed to grow abundantly while floating in liquid cultures. Unlike the GroEL2 protein, a general “housekeeping” chaperone that helps the cell's proteins fold properly, GroEL1 has a more specialized role. Without it, the mycobacteria could not construct mature, textured biofilms.

To find out how a chaperone protein might influence different growth phases, Ohja compared proteins made by mycobacteria strains with and without GroEL1. They showed that without the chaperone, the cells were lacking a key part of their fatty acid synthesis machinery. Then the group compared the fatty acids profiles of the two strains. The bacteria without GroEL1 made less

fatty acid in general and none of the particular mycolic acids required to produce a biofilm.

“These studies emphasize that fatty acid synthesis is a highly regulated process that depends on the physiological growth state of the cells,” said Hatfull. Researchers must do further study to find out how the chaperone causes the change in mycolic acid production, he said, but it is likely that it throws a molecular switch in the synthesis machinery.

Mycobacterium tuberculosis also has two *GroEL* genes, and its GroEL1 protein is 90 percent identical to the *M. smegmatis* GroEL1. Even though there is no direct evidence yet that *M. tuberculosis* forms biofilms, Hatfull and Jacobs say it is highly likely that the two GroEL1 proteins act in similar ways to change mycolic acid synthesis—a hypothesis they plan to test next. The same mechanism might also be at work in *M. ulcerans* and *M. leprae*, which both cause painful, disfiguring diseases.