PROFILE

Profile of William R. Jacobs, Jr.

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The expression on the young man's face as he stood by the railing surrounding the zebra enclosure at the Bronx Zoo might have been mistaken for awe at the animals' magnificent hides or spry gaits. However, as the man reached into the enclosure and scooped a clump of soil and zebra dung into his hands, he had something different in mind. The soil harbored bacteriophages, bacteria-infecting viruses that have shaped the life's work of William "Bill" Jacobs, Jr., a professor of microbiology, immunology, and genetics at Albert Einstein College of Medicine of Yeshiva University in New York City, an Investigator of the Howard Hughes Medical Institute, and a member of the National Academy of Sciences.

Since the mid-1980s, Jacobs has used phages to develop gene-transfer systems for *Mycobacterium tuberculosis* that have allowed researchers to identify tuberculosis (TB) drug targets and their mechanisms of action, study the virulence mechanisms of *M. tuberculosis*, and distinguish drug-sensitive from extensively drug-resistant strains. In the coming years, Jacobs hopes to introduce a multivalent vaccine capable of protecting against TB, HIV, and malaria. His Inaugural Article describes the point mutation in a bacterial strain that allowed researchers to clone genes and study drug targets (1). Jacobs' tenacity has earned him the nickname "TB Terminator" and cumulated in numerous awards, more than 300 publications and, in 2013, election to the National Academy of Sciences.

Humble Beginnings

Jacobs grew up with three sisters, including a twin, in the working-class town of West Mifflin, Pennsylvania, five miles from the steel mills that blanketed the area in smog (2). His father was a steelworker; his mother was a homemaker who was troubled by her son's tendency to stumble into the coffee table. "She just knew something was different," Jacobs says. "As soon as I started going to the eye doctor at four and a half, they could see the damage in my eyes from the retinitis pigmentosa."

Most people with the degenerative eye disease are blind by the time they reach middle age. "I see through a tunnel that is continuing to shrink," Jacobs says. "If you look straight ahead, you can probably see your hands about 90 degrees off center. But when I was growing up, I could only see about 20 degrees off center." These days, it is around 6 degrees. "If I were to look at your left eye, I would not see your right eye or your mouth."

As a young child, Jacobs knew he would be a scientist. "I grew up during the Sputnik era," he recalls. "When John Glenn went to the moon, I wanted to be an astronaut. But because of my eyes, I've never been able to see the stars."

An experiment in ninth grade inspired his first poster presentation at the Pennsylvania Junior Academy of Sciences and triggered a life-long passion for research. "I had this amazing molecular biology teacher, Frank Napier, who had us do research projects," he says. Jacobs opted to explore the effects of gibberellic acid, a plant growth hormone found in tomatoes and corn, on a single-cell alga called chlorella. After high school, Jacobs accepted a scholarship to the University of Pittsburgh in exchange for work as an equipment manager for the football team. However, Jacobs soon became so entrenched in athletics that he was neglecting studies, and he later transferred to nearby Edinboro State College for a fresh start. Jacobs remembers his days at the state school fondly. "At the time, I didn't even know about Harvard or Stanford so I went to a small state school, and I became a math major, where I had dedicated teachers and lots of opportunities to take elective courses in chemistry, physics, and biology. It was wonderful." A professor named Ellis Kline taught Jacobs' bacterial genetics laboratory. "Ellis taught us to isolate leucine auxotrophic mutants of *Escherichia coli*, and he used to pay for the amino acids out of his own pocket. I was hooked! I wanted to be a bacterial geneticist. About six of us who took that course have gone on to become full professors."

From Leprosy to TB

Jacobs graduated in 1977 and began applying to doctoral programs. "A lot of schools I applied to wouldn't even answer my letters," he recalls. Fortuitously, Jacobs was offered an interview at the University of Alabama, Birmingham. "Claude Bennett was the chair of the department. But I ended up being mentored by Roy Curtiss, III and Josie Clark Curtiss." Jacobs was accepted into the doctoral program at the University of Alabama, and his experience in the Curtiss laboratory ignited a love of teaching.

William R. Jacobs, Jr. Photo courtesy of Paras Jain.

In the decade before Jacobs began working with *Mycobacterium leprae*, researchers had discovered a way to cultivate the pathogen: not in artificial media, as most other pathogens are grown, but in nine-banded armadillos. "Charles Shepard and his collaborators at the CDC [Centers for Disease Control and Prevention] took biopsies from lepers, put them into nine-banded armadillos, and waited two years for the *M. leprae* to grow. Then Josie and I made the first genomic libraries for leprosy bacillus," he says. Jacobs had plenty of time to plan the construction of these libraries as he waited long months for the *M. leprae* to grow. "I used to have to make my own DNA ligase, cloning enzymes, and lambda packaging mixes for the construction of cosmid libraries."

This is a Profile of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 13264.

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Cosmids—bacterial plasmids engineered to express the packaging sequence of bacteriophage lambda—allow researchers to clone large fragments of DNA, reducing the number of clones per library and increasing the efficiency with which *M. leprae* DNA moved into *E. coli*. "I came to understand that phages were the most efficient way to get DNA into bacteria." Although he succeeded at making the cosmid libraries, *M. leprae* DNA was not expressed well in *E. coli*. Eventually, he managed to express *M. leprae* genes in *E. coli* under the control of a gene promoter from another bacterium, *Streptococcus mutans*. The findings were published in PNAS in 1986 (3).

After years of graduate research on *M. leprae*, Jacobs turned his attention to *M. tuberculosis*, the causative agent of TB. "TB was a much bigger problem than leprosy, and I knew no one yet had been successful in transferring genes into *M. tuberculosis*." Barry Bloom, a cellular immunologist at Albert Einstein College of Medicine, wanted to convert bacillus Calmette–Guérin (BCG)—the live attenuated TB vaccine currently given to children at birth—into a recombinant vaccine vector by augmenting it with genes from other pathogens, yielding a vaccine that protected against TB as well as other infectious diseases. "His idea was that if we could get genes from HIV and malaria and put them into BCG, we'd have a multivalent vaccine that would protect against all three." Both ideas, the recombinant bacillus Calmette–Guérin vaccine and gene transfer into *M. tuberculosis*, required the introduction of foreign DNA into mycobacteria.

Jacobs wanted to help. "It was amazing to me that in 1985 no one had yet been successful in transferring genes into *M. tuberculosis*, despite the fact that Robert Koch had defined the requirements for establishing the basis for an infectious disease using the tubercle bacillus over 100 years earlier." Molecular Koch's Postulate was difficult to achieve for *M. tuberculosis*, because of the danger of working with the organism and the fact that it took 20–30 days to generate a colony from a single cell. The Association of Public Health Laboratories warns that there is no safe level of exposure to *M. tuberculosis*. Researchers must be shielded by biohazard gowns in biosafety level 3 laboratories equipped with double-entry doors and specialized hoods. Jacobs' work with the organism, despite his deteriorating eyesight, hints at his determination to solve stubborn problems. TB is one such problem. "It seemed only logical that I should use phages from mycobacteria to develop gene-transfer systems."

When Jacobs arrived at Einstein in 1985 for a postdoc in Bloom's laboratory, he had decided he would use mycobacteriophages, but he needed starting material. "My first week here in the Bronx, I sent away for bacteriophages—but then I realized I could isolate them directly from soil." So he scooped a pile of dirt from his backyard and brought it to the laboratory. There, Jacobs mixed it with a strain of *Mycobacteria smegmatis*, named mc^{2}1, the first in his now-expansive collection of strains named in honor of Albert Einstein. "*M. smegmatis* is a fast-growing mycobacterium, and a great host strain for isolating mycobacteriophages as plaques," he says. Armed with the host strain, Jacobs isolated Bxb1 from his backyard in the Bronx and, later, Bxz1 from soil in the zebra enclosure at the Bronx Zoo.

Jacobs joined the faculty of Albert Einstein in 1986 as an associate scientist in the department of microbiology and immunology. His initial goal was to achieve plasmid transformation for mycobacteria. Although plasmids had been isolated from other mycobacteria, attempts to make *E. coli* mycobacteria shuttle plasmids had been unsuccessful. "I reasoned that transfection of purified mycobacteriophage DNA into mycobacteria and assaying for plaque formation provided a rapid assay for DNA entry. Moreover, I hypothesized it should be possible to develop a novel vector, which I named a 'shuttle phasmid,' that could replicate in *E. coli* as a plasmid and in mycobacteria as a phage."

The shuttle phasmid allowed for the introduction of foreign DNA into mycobacteria (4). Furthermore, shuttle phasmids provided the tools to demonstrate that failed transformation was not a result of restriction of *E. coli* DNA by mycobacteria, and allowed Jacobs to establish a selectable marker gene and selection system. Focusing on optimization of DNA transformation in mycobacteria resulted in three transformants after many failed attempts.

"We hypothesized that perhaps we had isolated a mutant of *M. smegmatis* that enabled plasmid transformation. This amazing mutant—named mc^{2}155, isolated by my first graduate student, Scott Snapper—transforms four to six orders-of-magnitude more frequently than its parent. It enabled us to isolate the previously unknown target of the TB drug isoniazid and develop plasmid expression vectors for recombinant BCG," Jacobs recalls.

In his Inaugural Article (1), Jacobs describes the mutation that enables mc^{2}155 to be transformed with plasmids. "After 24 years, it turns out it's a mutation in a gene encoding a Structural Maintenance of the Chromosome protein, a protein found in all life forms from archaebacteria to man," he says.

The *M. smegmatis* strain mc^{2}155 became a workhorse for mycobacterial research and the isolation of new mycobacteriophages. In 1988, Graham Hatfull from the University of Pittsburgh visited the Jacobs laboratory and determined the DNA sequences of Jacobs' mycobacteriophages, including L5, Bxb1, TM4, and Bxz1, the phage Jacobs isolated from the Bronx Zoo.

A few years later, Jacobs' twin sister, a high school biology teacher, asked if Jacobs had any science project ideas for her students. Jacobs put his sister in touch with Hatfull, who arranged a "phage hunting" expedition. Mycobacteriophages were isolated from places as diverse as the Bronx Zoo, rose gardens, and barnyards by established researchers, local high school students, and Zulu children in South Africa. "We ended up publishing a paper in *Cell* that not only got the cover, but the first and only centerfold ever: exposing the naked genomes of 14 new viruses. On the byline: Pelham Memorial High School in New York and Latrobe High School in Pennsylvania," he recalls (5).

Six years ago, Jacobs started a phage-hunting safari in South Africa. "I was thinking, how am I going to get those Zulu kids excited about science?" he recalls. The first year, Jacobs invited 11 students from the University of Kwazulu-Natal to a game park and encouraged them to take soil samples from around the park. "The first student to isolate a phage in our course that year was a young lady, Lindokuhle Ndlandla, that grew up in that Zulu village. She was the first woman in her village ever to go to college. She's finished her master's, and she's doing a PhD at the University of Pretoria in South Africa." Last year, the program hosted 33 students.

The Elusive Multivalent Vaccine

Since Jacobs arrived at Einstein, he has shared Bloom's vision of making a multivalent TB vaccine. Jacobs and others have attempted the multivalent vaccine several times with limited success. However, he keeps trying. "Traditional BCG clearly doesn't work," he says. "It's still given to half the world's population within four hours of being born to two weeks. It protects against the more severe forms of TB, but it doesn't really protect adolescents. It might reduce severity of TB for children at birth, but we still haven't figured out how to get a sterilizing immunity against TB."

In the meantime, Jacobs and collaborators elucidated the mechanism of action of isoniazid (6–8), determined how to attenuate TB (9, 10), determined why the bacillus Calmette–Guérin vaccine was attenuated (11, 12), determined how to create better vaccines (13–15), revealed why mycobacteria stain acid fast (16), and uncovered a strategy for creating auxotrophic mutants: strains of bacteria that are incapable of making a particular compound required for their growth. In addition, the shuttle phasmids have provided the tools for efficient transposon mutagenesis (17) and specialized transduction (18) of *M. tuberculosis*, techniques that Jacobs and his collaborators are exploiting to systematically knock out every gene in *M. tuberculosis* in a high-throughput fashion.

In 1993, Jacobs developed a rapid method for using the luminescent reporter phages to determine the drug susceptibilities of *M. tuberculosis* (19). "Having the tools of genetics, in my mind, is just logical mathematics," he says. "When you work with TB, it takes a month to grow up the strain and a month to do drug susceptibilities. So I took the gene from fireflies, the luciferase gene, and put it into this phage. Now, when I add this phage to TB, it makes it glow like a lightning bug."

In recent years, Jacobs has once again focused his energy on the development of a multivalent vaccine. "We've had mixed success. It's something we continue to work on," he says. "I think a lot of the problem is that we did have stable BCG/HIV constructs that we think are going to be very good for a priming response in the HIV vaccine development, but we've just recently figured out how to do that very well. The promise hasn't yet been realized."

Looking Ahead

The greatest single impediment to the eradication of TB, Jacobs says, is the persistence of a fraction of *M. tuberculosis* cells in a population. The Jacobs laboratory recently (20) showed that both actively growing cells and persisters that do not respond to single bacteriocidal drugs can be rapidly sterilized by vitamin C. "The so-called 'persister cells' are refractory to TB drugs or immune effectors. We need to develop ways to either kill persistent *M. tuberculosis* cells or prevent their formation," he notes.

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