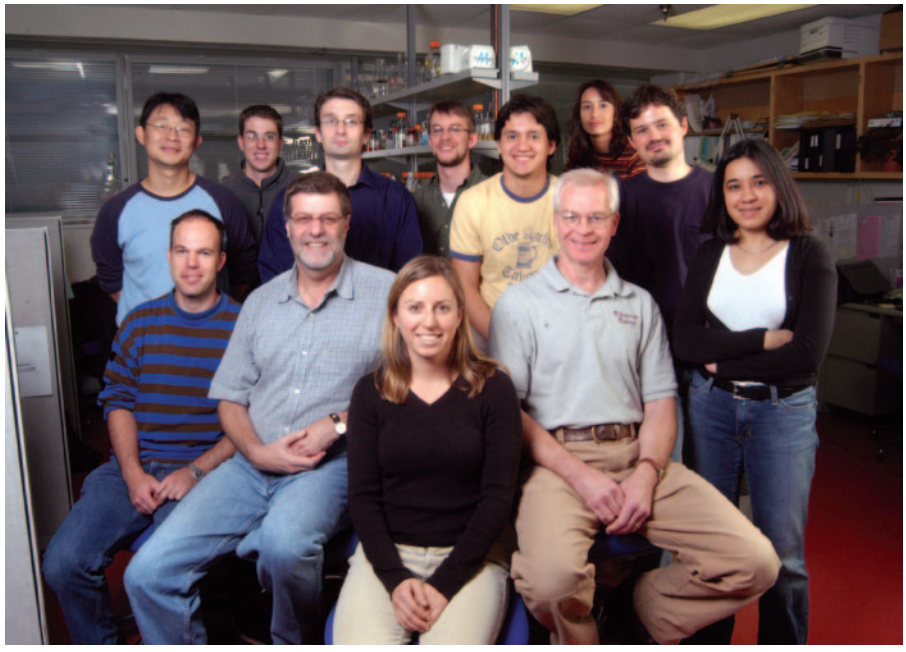
Biography of E. P. Greenberg

For the bacterium *Pseudomonas aeruginosa*, communication is a powerful tool. Twenty years ago, the idea that bacteria communicate with each other was not widely accepted. Today, bacterial communication is on the forefront of understanding devastating infections, such as those that plague cystic fibrosis patients or burn victims. E. Peter Greenberg, elected to the National Academy of Sciences in 2004, first introduced the term "quorum sensing" in 1994 to describe the regulation of bacterial gene expression by means of population sensing (1). Today, quorum sensing is proving more and more complicated. In his Inaugural Article on page 15833 in this issue of PNAS (2), Greenberg presents new research that questions the traditional paradigms of promoter recognition by the transcription factors involved in quorum sensing.



The Greenberg laboratory group at the University of Iowa. Back row, from the left: Joon-Hee Lee, Keith Brady, Yannick Lequette, Breck Duerkop, Luis Caetano-Antunes, Kimberly Lee, Martin Schuster, and Sudha Chugani. Seated, from the left: Ehud Banin, E. P. Greenberg, Esther Volper, and Mark Urbanowski.

Leading a Student to Water

For the past 16 years, Greenberg has been Sheppard Endowed Professor of Microbial Pathogenesis at the University of Iowa (Iowa City). He plans to move to the University of Washington (Seattle) in January 2005 to become chair of the microbiology department. Born in New York City in 1948, Greenberg's family moved to the west coast when he was a toddler, and he attended high school in Seattle. At the time, Greenberg says, "I was a high school student who was not engaged in academics." But his high school biology teacher Mr. Leuthey, whose first name now escapes Greenberg, "encouraged us to try our hands at research even though we were untrained." Leuthey set up an overnight field trip to the Washington coast for a first-hand look at marine life. On the trip, Greenberg ended up hooked on the world of invertebrates: "For the rest of high school, at least I paid attention in biology." Upon graduation, however, Greenberg still was not in love with academics. This changed during his year at Everett Junior College, where he doubled his grade-point average and discovered that "teaching wasn't about seeing how hard someone could work at a mindless task. It was about conveying interesting information."

In 1966, Greenberg entered Western Washington University (Bellingham, WA): "By then I was head over heels for biology." His advisor, Don Williams, "told me that it would be much more fulfilling for me to do experimental science instead of observational invertebrate ecology." Williams invited him to do a project studying the effects of heavy metals on mitochondrial respiration: "That meant I had to get mean rats out of cages, and rats have teeth." Around the same time, he took a summer course in microbiology with Don Schwemmin. "I realized that microbes respired," he says. "And they don't bite." Up to that point, Greenberg planned to attend graduate school in biochemistry, but instead he decided on microbiology and submitted last-minute applications. Despite his interest in marine invertebrates, Greenberg began a medically oriented doctoral program at the University of Iowa. Greenberg stayed for 2 years and earned a master's degree before transferring to a program more focused on basic microbiology at the University of Massachusetts in Amherst.

At the University of Massachusetts, Greenberg worked with Ercole Canale-Parola, then one of the few experts on free-living spirochetes. Most of the research interest at the time focused on the spirochete species that cause human diseases such as syphilis. Greenberg, still fascinated by his experience with the coastal invertebrates, wanted to study marine species. Canale-Parola sent him to meet with Holger Jannasch at Woods Hole Oceanographic Institute (Woods Hole, MA). After collecting marine mud, Greenberg set about isolating spirochetes. He had the opportunity to pursue further enrichments when Bob Hespell, another student, "handed over the mud" he had collected from a solar pond in Israel. Although the high-salt medium was difficult to make, Greenberg found a new spirochete species that flourishes in high salinity (3). For his thesis, he studied chemotaxis, investigating chemicals to which spirochetes respond (4). "My college advisor had it right," he recalls. "I had an interest in finding unique things and then doing experiments."

Bacterial Chatter

During the summer of 1973, Greenberg attended a microbiology course at Woods Hole that Canale-Parola and Jannasch had recommended. Greenberg remembers a group of 15 students, "all people like me, relishing the idea that we wouldn't be graded. We were there to absorb information. We had 8 weeks to try and move some science forward." Greenberg learned how to be efficient, but he also gained more than laboratory

This is a Biography of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 15833.

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skills. "I learned not to be embarrassed by enthusiasm for working in the lab," he says. While there, Greenberg met instructor Ken Nealson, who was finishing postdoctoral work with Woody Hastings at Harvard University (Cambridge, MA). Nealson recently had found that an unidentified molecule accumulated in culture and acted as an autoinducer for the production of light in *Vibrio fischeri*, a marine bacterium (5). "To me, it sounded like communication in bacteria," Greenberg recalls. Even though it was only his first year of Ph.D. studies, Greenberg thought, "Now I know what I want to do my postdoc on." As for behavior in bacteria, "most people thought it was nonsense that bacteria could communicate." When Greenberg finished his degree in 1977, he started a postdoctoral position in Hastings' laboratory to study bacterial communication.

After a year, Greenberg was recruited to an assistant professorship at Cornell University (Ithaca, NY). He established two research programs, making spirochete motility his priority and putting bacterial communication on the back burner. The *V. fischeri* research picked up steam, however, and he later found a local collaborator in Anatol Eberhard, at Ithaca College (Ithaca, NY), who had identified the chemical signal from *V. fischeri* as an acyl-homoserine lactone (6). Greenberg's first graduate student to work on the project, Heidi Kaplan, set about finding how the autoinduction signal enters and leaves cells. No "elaborate bells and whistles" were found, as Greenberg had expected. Kaplan found that the signal is transmitted by means of passive diffusion, and that very simplicity makes it a quorum-sensing molecule (7). As Greenberg puts it, "Accumulation in the environment is reflected by intracellular accumulation." Quorum-sensing bacteria excrete signal molecules that accumulate in the environment. When the population reaches a certain density, it can influence the environmental signal concentration and thus the cellular concentration. In *V. fischeri*, the signal activates the transcription of luminescence genes, among others.

The term quorum sensing was not coined until 1994 when Greenberg, Clay Fuqua, and Steve Winans wrote a review (1). "We had to come up with a really good title or no one would read it," says Greenberg. Winans suggested the term after discussing the process with a relative who said that the bacterial phenomenon sounded like waiting for a quorum at a business meeting. Thus, the group decided to call it quorum sensing: "The term really caught on and somehow crystallized a field of people."

Changing Course

In 1988, Greenberg and his wife, Caroline Harwood, whom he had met at the University of Massachusetts, moved to the University of Iowa for professorships. Greenberg continued both the *V. fischeri* and spirochete research there. With *V. fischeri*, he focused on LuxR, the transcription factor activated by the autoinducer. As more transcription factor genes were sequenced, Greenberg noticed that the C-terminal sequence of LuxR had a conserved region, whereas the N-terminal sequence appeared to be variable (8). He surmised that the C-terminal sequence was used for binding DNA, whereas the N-terminal sequenced bound to signal. One of his graduate students, Sang Ho Choi, placed different deleted versions of the LuxR gene in *Escherichia coli* and analyzed which sequences bestowed function (9–11). Choi and Greenberg found that 30% of the C-terminal sequence retained the ability to activate luminescence genes, and signal was not required for activation. Another graduate student, Brian Hanzelka, showed that the 60% closest to the N terminus of LuxR could bind the signal but could not affect gene transcription (12).

"Good ideas in the biological world are not restricted to the rare example."

As all of these mechanistic details became clear, Greenberg thought it was time to stop spirochete study and focus on signaling. In 1991, he told a colleague, Phil Matsumura, that he was not going to continue his chemotaxis work. Matsumura said, "Pete, you're crazy. Everyone in chemotaxis knows you." But Greenberg decided he had to "take the risk and follow my nose." He sensed that there was much more to be discovered regarding *V. fischeri* and that "good ideas in the biological world are not restricted to the rare example." A serendipitous moment came soon after. Greenberg spoke with Charlie Cox of Barbara Iglewski's laboratory at the University of Rochester (Rochester, NY), who told Greenberg of a virulence gene regulation discovery in *Pseudomonas*. An unpublished sequence of one of the bacterium's genes showed that its closest relative was *luxR*, the same quorum-sensing gene that Greenberg was studying in *V. fischeri*. "Wow," thought Greenberg. "We didn't quite know what to make of it, but Iglewski and I were smart enough to begin a collaboration."

Communication Strategies

As the *Pseudomonas* story unfolded, it became clear that quorum sensing controlled many genes that were important in virulence. Greenberg began to try to find which *Pseudomonas* genes were controlled by quorum sensing. "We now know that about 300 of the 6,000 genes are controlled by quorum sensing," he says (2, 13). But the pattern is not straightforward and appears to be highly complicated (2). "Microarrays revealed patterns of expression we never would have expected," says Greenberg. Different genes were found to respond differently to the two autoinducer signaling molecules in *Pseudomonas*. Some genes respond to either one or the other, but some respond to both with varying specificities. Of the 300 genes, 250 require signal for activation, but they do not respond to signal immediately (14). Initially, Greenberg believed there would be no product released until quorum was reached, at which point the quorum-controlled genes would be activated together, but he and his researchers have found that there is a continuum of staggered responses (14).

To determine the reasons for the different gene responses, Greenberg and his team purified the *Pseudomonas* transcription factor LasR. "LuxR homologs are notoriously difficult to purify," he says. With LasR in hand, they could now start to understand how it recognizes promoters. LasR controls dozens of genes, each one slightly differently. Greenberg's Inaugural Article in this issue (2) is the first article detailing how LasR interacts with promoters. "We thought we knew what LasR-dependent promoters looked like," he says, but now with *in vitro* study, this does not appear to be the case. LasR appears to be able to bind even without a recognizable las box, a 20-bp inverted repeat thought to be required for binding (2). "We have to reimagine what a binding site looks like," says Greenberg. He plans to continue work that will allow the grouping of LasR binding sites into families.

In the meantime, Greenberg also has research interests beyond the basic biology that hooked him early on. "We knew from the beginning that quorum sensing is controlling virulence gene expression in *Pseudomonas*." This "looked like the Achilles' heel" for a major opportunistic pathogen. *P. aeruginosa* infections are the primary cause of death in cystic fibrosis patients. The bacteria

form biofilms inside the lungs that block airways (15, 16). The large population of bacteria is protected from eradication by antibiotic treatment and the immune system inside the biofilm. Greenberg hopes that one day it will be possible to develop inhibitors of quorum sensing to use as antivirulence agents to block communication (17): "The principle is untested because there is no drug to test yet. Can a drug that does not kill bacteria or even inhibit their growth in a test tube block virulence in the host?" He believes that the knowledge exists to develop more effective drugs to treat chronic *Pseudomonas* infections but that the drug industry currently has little interest in it. These beliefs have become a passion for Greenberg. In deciding to move to Seattle, Greenberg believes that the area offers the perfect mix of resources to address this issue. He cites Seattle as the home to the Cystic Fibrosis Therapeutics Development Network, an active biotechnology industry, and "excellent *Pseudomonas* genomics people." Says Greenberg, "A pet project of mine will be trying to find the money to coordinate all of this talent, focus it on the specific problem of chronic *Pseudomonas* infections, and see if we can't make good things happen."

Greenberg has mixed emotions about heading to the University of Washington to chair the microbiology department. On the one hand, he will be returning to the Pacific Northwest where he first became enthralled by science; on the other, he is "leaving a really good thing behind." But once again, he is following his passion for research, which has permeated his life: "My profession is my hobby. Some people are horrified by that, but I can't help it, and I think I am among the fortunate few."

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