

Profile of Bonnie L. Bassler

The Agouon Institute in La Jolla, CA, sits on a cliff overlooking the Pacific Ocean. On clear nights, plumes of bioluminescent bacteria, squid, and other organisms drift through the opaque water. “It’s like staring at the constellations, only much more intense,” says Princeton University (Princeton, NJ) molecular biologist Bonnie L. Bassler. “The waves glitter. Boat wakes glitter. Your footprints glitter if you walk along the beach.”

Bassler spent 4 years as a postdoctoral fellow at Agouon, which was founded in 1978 by a small grant from the Office of Naval Research (ONR). She studied the glow-in-the-dark bacteria that cause the oceanic light show. Her work hinged on earlier research demonstrating that these bacteria only gave off light when there were enough of their cohorts around to make it worth the effort. The bacteria kept track of their numbers—quorum sensing—by using chemical signaling molecules called autoinducers.

Over the past two decades, Bassler’s research has teased apart how glow-in-the-dark, or bioluminescent, bacteria and other bacterial species communicate with autoinducers. It turns out that bacteria use these chemical messengers in ways reminiscent of the ways humans use language—to organize for a common good, to warn each other about potential dangers, or even to deceive others. Until this research, says Bassler, “there was this general feeling in the scientific community that only higher organisms had intercellular communication.”

The MacArthur Foundation awarded Bassler its prestigious fellowship in 2002, lauding her for her contributions to understanding the bacterial lexicon. She was elected to the National Academy of Sciences (NAS) in 2006.

Her inaugural article in PNAS shows that four small strands of RNA regulate quorum sensing and virulence, the ability of a microorganism to cause disease, in *Vibrio cholerae*, the bacteria that caused cholera epidemics around the world (1). Small RNAs play a significant role in eukaryotic embryonic development, further supporting Bassler’s observations on the complexity of bacterial life.

Bacteria Were Not Important

Bassler was born in Chicago, IL, but grew up in Danville, CA, due east of San Francisco. Intellectually curious from a young age, she enjoyed solving logic problems, putting puzzles together, and “trying to figure out the end of a



Bonnie L. Bassler

book before you got to it,” she recalls. “I also loved animals and really thought I wanted to be a veterinarian,” she says from her office on the “the lower-life-forms floor” of the Lewis Thomas Laboratories at Princeton.

She enrolled at the University of California (UC), Davis (Davis, CA) with the intention of majoring in veterinary sciences. However, dissection didn’t mesh with her constitution—“I’d pass out,” Bassler admits—and neither did the heavy emphasis on memorization in her anatomy classes.

Instead, biochemistry and genetics became her gateways to solving biological puzzles. She found a mentor in UC Davis biochemistry and molecular medicine professor Fredrick Troy. He was working on two projects: one on bacterial carbohydrates and the other on the Epstein-Barr virus and its relationship to cancer. The cancer project called to the young scientist. “I wanted to do something important, and bacteria were not something important,” Bassler says of her belief at the time.

Contrary to her wishes, Troy put her to work characterizing an enzyme in *E. coli*, neuraminidase (2). The enzyme cleaves sugars from membrane glycoproteins, alterations of which have been implicated in neural cell adhesion and bacterial meningitis.

At first, Bassler was livid for having to work on bacteria. But she soon fell in love with the prokaryotes. “Everybody thinks they’re the simplest organisms in the world, but I think they’re the perfect creatures to work on,” she says. “They do such miraculously sophisticated things. I was 19 then, and I have never worked on anything else.”

After applying to graduate programs in biochemistry, Bassler crossed the country to attend Johns Hopkins University (Baltimore, MD) on Troy’s advice. There, she continued to study bacterial-carbohydrate interactions in Saul Roseman’s group. Roseman had just received a grant from the ONR to investigate bacteria that adhere to surfaces. Called “biofilms,” these mixed consortia of bacterial species slowed down Navy vessels, corroded sensitive equipment, and cost time and money to remove. “Roseman handed me this tube of bacteria and said, ‘This is your project,’” she recalls. “It was a marine *Vibrio*, *V. furnissii*, and I started to work on how they adhered to sugars.” Gram-negative bacteria with a curved, rod shape, *Vibrio* typically inhabit saltwater areas and include several species, such as *V. cholerae* and *V. vulnificus*, which cause death and disease in humans.

V. furnissii, Bassler observed, was strongly attracted to certain carbohydrate substrates such as *N*-acetylglucosamine monomers and oligomers. These complex sugars form the shells of many microscopic organisms (3). “It turned out that the bacteria would swim—they would chemotact—to, adhere to, and eat these sugars,” says Bassler.

Genetics for Dummies

Chemotaxis in *V. furnissii* heightened Bassler’s curiosity about the nature of bacterial perception. As luck would have it, the ONR held a conference in Baltimore as she was finishing her doctorate. The conference brought together many investigators with similar naval grants; one of those researchers was geneticist Michael Silverman of the Agouon Institute.

Silverman, now retired, had been studying a phenomenon first described decades earlier by NAS member and Harvard University (Cambridge, MA) professor J. Woodland Hastings. Hastings discovered quorum sensing in the bioluminescent marine bacteria *Vibrio fischeri* and its relative *Vibrio harveyi* (4). He coined the term “autoinducer” to describe the chemical messenger, a homoserine lactone (HSL), that the microorganisms used to communicate (5, 6).

At Agouon, Silverman identified the signaling cascade that controls quorum sensing in *V. fischeri* (7–9). He showed

This is a Profile of a recently elected member of the National Academy of Sciences to accompany the member’s Inaugural Article on page 11145 in issue 27 of volume 104.

© 2008 by The National Academy of Sciences of the USA

that a protein called LuxI produced the autoinducer molecule. A receptor, LuxR, bound the autoinducer and activated transcription of the genes encoding the enzyme luciferase. This enzyme catalyzes light-emitting reactions in diverse organisms, from bioluminescent bacteria to mushrooms, worms, and of course, fireflies.

Bassler's encounter with Silverman at the ONR conference was a stroke of luck. The reclusive geneticist hadn't given a talk in more than five years and wouldn't give another for nearly five more.

"It was the first meeting I had ever attended," Bassler reveals. "Mike had just figured out the nub of the molecular mechanism of what we now call quorum sensing. He had chopped up the [*V. fischeri*] chromosome, put it into *E. coli*, and produced bacteria that made light, made the signal molecule, and only turned on light at high cell numbers. He figured out the whole thing in this one experiment." She remembers him saying, "Don't you see, these bacteria are communicating with this molecule. They are acting multicellular."

Bassler, who wanted to add genetics to her scientific repertoire, was captivated by the ease of genetically manipulating bioluminescent organisms. "You can get the results by turning off the lights and seeing if the bacteria glow or don't glow; what could be easier?" she says. "It's genetics for dummies."

At the end of Silverman's 20-minute presentation, Bassler raced up to the podium and asked for a job. "I thought, 'I have to work on this or I'm going to quit science!'" she jokes. "And for reasons that I've never understood—he didn't even know who I was—he gave me a postdoc position." Bassler returned to the West Coast and began studying quorum sensing in *V. harveyi* at Agouron.

Bacterial Esperanto

During her postdoctoral fellowship, Bassler defined the quorum-sensing circuit of *V. harveyi*. She showed that, like *V. fischeri*, *V. harveyi* communicated with other members of its species by using an HSL autoinducer (10). In addition, Bassler discovered that *V. harveyi* had more than one molecule for quorum sensing; she called this additional molecule autoinducer-2 (AI-2), and the original, found by Hastings, became autoinducer-1 (AI-1).

Soon after her discovery of AI-2, in 1994 Bassler decamped the La Jolla institute for a tenure-track position at Princeton. By this time, and thanks to Silverman's and Bassler's research, the field of quorum sensing had gained traction within the scientific community.

The pathways identified in *Vibrios* and in other bacteria painted a broader picture of bacterial communication, one that looks to Bassler like development in higher organisms.

"It's not just bioluminescence," she explains. "*V. harveyi* turn on and off hundreds of genes using quorum sensing. The communication process initiates an enormous genetic program analogous to development in embryos, and it allows the bacterium to make the change from acting as an individual to acting as a member of a group."

At Princeton, Bassler's research on quorum sensing revealed a major surprise: Bacteria could not only talk to members of their own species, but they also used AI-2 for interspecies communication (11). Her group identified a family of highly homologous genes called *luxS*, which are present in hundreds of species of bacteria, including *E. coli* and *Salmonella typhimurium*. She showed that these dissimilar bacteria produced AI-2 and that AI-2 from *E. coli*, for example, could activate quorum sensing and bioluminescence in the unrelated *V. harveyi* bacterium.

"You can get the results by turning off the lights and seeing if the bacteria glow—it's genetics for dummies."

The idea that the planet's simplest organisms could speak with one another by using a common molecular language—"bacterial Esperanto," as Bassler calls it—was so revolutionary that the MacArthur Foundation gave her one of its coveted fellowships, anointing her with the "genius" tag.

The professor of molecular biology at Princeton is unromantic about her accomplishments. "It seems so obvious to me now," Bassler says. "Of course bacteria can speak between species. They live in these unbelievable mixtures and need to know who their neighbors are—it's information useful to their survival."

In 2005, Bassler became a Howard Hughes Medical Institute Investigator. Bassler credits the Institute for "revolutionizing how [her lab] does science. They've given me the resources to fully unleash the imaginations of my students and postdocs."

Biological Boron

Although Bassler's research at Princeton proved that many bacterial species produce AI-2 and use it for communication, the molecule itself was far from easy to characterize. Further work in her laboratory provided an answer: As it turns out, AI-2 is a collection of interchangeable molecules, all of which have a similar chemical composition but different three-dimensional structures.

The LuxS protein found by Bassler catalyzes the formation of a five-carbon ketone, 4,5-dihydroxy-2,3-pentanedione (DPD); this pentanedione spontaneously converts into a set of different cyclic molecules, the entire collection of which is known as AI-2 (12).

Still, the question remained, which of these molecules did bacteria use to communicate? Bassler teamed up with Princeton crystallographer Fred Hughson to find the answer. In an elegant experiment, Hughson trapped *V. harveyi*'s AI-2 molecule inside its receptor, LuxP, and deciphered the structure of both the receptor and the autoinducer (13).

Bassler says that this was "the first time anyone had solved the structure of a small molecule by trapping the ligand inside its receptor. We solved the whole structure of this receptor, which we couldn't care less about, just to get the autoinducer molecule."

The crystallography experiment revealed another surprise: AI-2 binds the element boron and uses it as a cofactor—an element needed by the AI-2 molecule to carry out its biological role.

Scientists have found few uses for boron in nature; it exists in only trace amounts in terrestrial environments. The oceans, however, have an abundance of the element. Thus, a boron cofactor for the ocean-dwelling *V. harveyi*'s AI-2 made sense, because the bacterium could easily find the element in its natural environment. In contrast, boron seemed highly improbable for terrestrial bacteria such as *E. coli* or *Salmonella*.

This discovery raised questions about AI-2's role as the common bacterial tongue. Hughson and Bassler brought in Princeton organic chemist Martin Semmelhack to help address the puzzling results. Together, they discovered that the key to "bacterial Esperanto" was DPD's ability to spontaneously rearrange (14). They proved that, although *V. harveyi*'s, *E. coli*'s, and *Salmonella*'s AI-2s were structurally different, and the latter two autoinducers didn't contain boron, each bacterium's AI-2 could interconvert and would be recognized

and “read” by the other species’ receptors.

Strategic Virulence

In bacteria, quorum sensing controls more than just bioluminescence. By cooperating with each other and with other species, bacteria can act like multicellular organisms. This cooperation has helped the “simple” prokaryotes colonize a wide range of environments from humans and animals to the searing thermal vents on the ocean floor and the sub-zero conditions beneath polar ice. But, as Bassler reminds us, bacteria are far from altruistic. “They cooperate because they have to in order to thrive,” she says. “They would certainly kill their neighbors or their hosts, if they could do it and get away with it.”

Many researchers have now shown that quorum sensing controls virulence in diverse bacteria (15–17). “It doesn’t do one bacterium any good to start secreting toxins because the host’s immune system would get rid of it,” says Bassler. “If they wait, count themselves using quorum sensing, and they all launch their attack together, it’s a fabulous strategy. It just depends on what side you’re on.”

For the disease-causing *V. cholerae*, Bassler’s lab found that three parallel quorum-sensing signaling channels control its virulence (18). Further work from her laboratory, in conjunction with Princeton theoretical physicist Ned Wingreen, elucidated the signaling pathway behind *V. cholerae*’s virulence mechanism. Four small, redundant RNAs act like a switch, regulating the production of the master quorum-sensing transcrip-

tion factor, HapR (19). Bassler’s Inaugural Article builds on this research and shows that these small RNAs control quorum sensing and virulence in *V. cholerae* strains that lack functional HapR proteins as well.

Therapeutic Potential from Lying Bacteria

Much like higher organisms, bacteria have evolved a variety of ways to use language, not all of them beneficial to other species. In mixed populations, commensal *E. coli*, Bassler observed, consumed AI-2 from both *V. harveyi* and *V. cholerae*, effectively fooling the other organisms into thinking there were fewer of their own species present (20). *E. coli* that live in the gut may use this mechanism to halt the spread of *V. cholerae* bacteria, which detach from the intestinal wall and move out of the body to infect other individuals by using a quorum-sensing mechanism.

Bassler’s work on AI-2 reveals a potential for the development of new classes of broad-spectrum antibiotics. Therapeutics that interfere with quorum sensing may provide ways of combating drug-resistant infections, relying on AI-2’s universality to do the dirty work. Surfaces prone to biofilm formation, such as heart valves, catheters, boat hulls, or teeth, could be coated with anti-quorum sensing molecules to prevent bacterial build-up. Bassler and Semmelhack are fabricating AI-2 antagonists that they hope will turn into lead molecules for such new antimicrobial therapies.

Bacteria, however, are not only our enemies, as Bassler’s research with commensal *E. coli* vividly demonstrates. Probiotics

that up-regulate quorum sensing mechanisms in these bacteria could help protect people from other prokaryotic invaders. Such compounds could also increase the production of industrial processes as diverse as cheese-making and fermentation to the production of antibiotics.

According to Bassler, “it is very clear that bacteria require quorum sensing to be virulent as well as to carry out many beneficial behaviors. It is also very clear that the world needs new therapies that are pro- or antibacterial. A very attractive idea is to manipulate the quorum-sensing conversation, to either shut bacteria down when they’re doing things that we don’t like, or beef up their conversation when they’re doing things we do like.”

Throughout her decade-and-a-half of eavesdropping on bacteria, through the lean years before the MacArthur fellowship, when the Princeton professor didn’t have a single National Institutes of Health (NIH) grant, Bassler has remained preternaturally energetic and upbeat. The best-educated aerobics instructor in the country (she teaches an early-morning class at the local YMCA) and erstwhile actress (her husband is an actor) says that “no one could be happier than me or have a more lucky life. You can’t have a better scientific career, and I can’t imagine having more fun than I’ve had.” She even managed to convince Silverman to come from California and join her for her NAS induction ceremony. He hadn’t flown in over a decade.

Farooq Ahmed, *Freelance Science Writer*

- Hammer BK, Bassler BL (2007) Regulatory small RNAs circumvent the conventional quorum sensing pathway in pandemic *Vibrio cholerae*. *Proc Natl Acad Sci USA* 104:11145–11149.
- Hallenbeck C, Vimr E, Yu F, Bassler B, Troy F (1987) Purification and properties of a bacteriophage-induced endo-*N*-acetylneuraminidase specific for poly-alpha-2,8-sialosyl carbohydrate units. *J Biol Chem* 262:3553–3561.
- Yu C, Bassler B, Roseman S (1993) Chemotaxis of the marine bacterium *Vibrio furnissii* to sugars: A potential mechanism for initiating the chitin catabolic cascade. *J Biol Chem* 268:9405–9409.
- Nealson KH, Platt T, Hastings JW (1970) Cellular control of the synthesis and activity of the bacterial luminescent system. *J Bacteriol* 104:313–322.
- Hastings JW, Nealson KH (1977) Bacterial bioluminescence. *Annu Rev Microbiol* 31:549–595.
- Nealson KH, Hastings JW (1979) Bacterial bioluminescence: Its control and ecological significance. *Microbiol Rev* 43:496–518.
- Engbrecht J, Nealson K, Silverman M (1983) Bacterial bioluminescence: isolation and genetic analysis of functions from *Vibrio fischeri*. *Cell* 32:773–781.
- Engbrecht J, Silverman M (1984) Identification of genes and gene products necessary for bacterial bioluminescence. *Proc Natl Acad Sci USA* 81: 4154–4158.
- Engbrecht J, Silverman M (1987) Nucleotide sequence of the regulatory locus controlling expression of bacterial genes for bioluminescence. *Nucleic Acids Res* 15:10455–10467.
- Bassler BL, Wright M, Showalter R, Silverman M (1993) Intercellular signalling in *Vibrio harveyi*: Sequence and function of genes regulating expression of luminescence. *Mol Microbiol* 9:773–786.
- Surette MG, Miller MB, Bassler BL (1999) Quorum sensing in *Escherichia coli*, *Salmonella typhimurium*, and *Vibrio harveyi*: A new family of genes responsible for autoinducer production. *Proc Natl Acad Sci USA* 96:1639–1644.
- Schauder S, Shokat K, Surette MG, Bassler BL (2001) The LuxS family of bacterial autoinducers: Biosynthesis of a novel quorum-sensing signal molecule. *Mol Microbiol* 41:463–476.
- Chen X, et al. (2002) Structural identification of a bacterial quorum-sensing signal containing boron. *Nature* 415:545–549.
- Miller ST, et al. (2004) *Salmonella typhimurium* recognizes a chemically distinct form of the bacterial quorum-sensing signal AI-2. *Mol Cell* 15:677–687.
- Dong YH, Xu JL, Li XZ, Zhang LH (2000) AiiA, an enzyme that inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of *Erwinia carotovora*. *Proc Natl Acad Sci USA* 97:3526–3531.
- Rothfork JM, et al. (2004) Inactivation of a bacterial virulence pheromone by phagocyte-derived oxidants: New role for the NADPH oxidase in host defense. *Proc Natl Acad Sci USA* 101:13867–13872.
- Guiral S, Mitchell TJ, Martin B, Claverys JP (2005) Competence-programmed predation of noncompetent cells in the human pathogen *Streptococcus pneumoniae*: Genetic requirements. *Proc Natl Acad Sci USA* 102:8710–8715.
- Miller MB, Skorupski K, Lenz D, Taylor RK, Bassler BL (2002) Parallel quorum sensing systems converge to regulate virulence in *Vibrio cholerae*. *Cell* 110:303–314.
- Lenz DH, et al. (2004) Parallel quorum sensing systems converge to regulate virulence in *Vibrio cholerae*. *Cell* 118:69–82.
- Xavier KB, Bassler BL (2005) Interference with AI-2-mediated bacterial cell-cell communication. *Nature* 437:750–753.