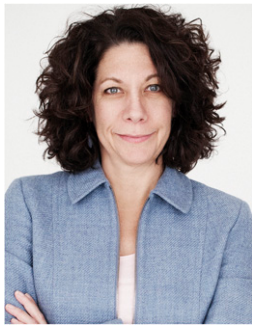
From Biochemistry to Genetics in a Flash of Light



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The Genetics Society of America (GSA) Medal recognizes researchers who have made outstanding contributions to the field of genetics in the past 15 years. The 2019 GSA Medal is awarded to Bonnie L. Bassler of Princeton University and the Howard Hughes Medical Institute in recognition of her groundbreaking studies of bacterial chemical communication and regulation of group behaviors.

I start by confessing that I've never taken a genetics class. Both my undergraduate and graduate degrees are in biochemistry. As an undergraduate, I spotted a billboard with a flier offering a lab internship with a team studying bacterial polysaccharide linkages. Wondering what lab work was like, I signed up. This random choice turned out to be monumental for me because it began my lifelong adventure working on bacteria.

I found lab research to be thrilling. Ruminating on career possibilities, I concluded that the best route to staying at the bench was to remain in school. As a graduate student, I studied chemotaxis to and degradation of chitin by the marine bacterium *Vibrio furnissii*. My project relied exclusively on biochemical approaches, primarily characterization of enzyme mechanisms and products of chitin polysaccharide catabolism.

As my graduate studies were wrapping up, I started to think about postdoc possibilities. I wanted to learn "genetics." Specifically, I wanted to use a restriction enzyme, something which seemed miraculous as a technique. The truth is, I was confusing molecular biology with genetics, both of which were utterly opaque to me. While I did not understand those approaches, I did know that I had a knowledge gap that was holding me back as a researcher. Around that time, I attended a conference devoted to marine biology. In one of the luckiest 30 minutes of my career, Michael Silverman explained how *Vibrio fischeri*, a bioluminescent marine bacterium, had the remarkable capacity to make light, but only at high cell density. He acknowledged Woody Hasting's foundational results demonstrating that *V. fischeri* produced and released a signal molecule, called an autoinducer, that accumulated with increasing cell density (Nealson *et al.* 1970). At high cell density, *V. fischeri* cells detected the autoinducer, and, in response, emitted bioluminescence. Silverman went on to explain his own, now landmark, strategy to discover the genes involved. He shotgun cloned *V. fischeri* genes into *Escherichia coli*, turned the lights off in the room, and identified recombinant *E. coli* colonies that glowed in the dark. The screen yielded the luciferase structural genes (*luxCDABE*) and two regulatory genes *luxI*, encoding the autoinducer synthase, and *luxR*, encoding the autoinducer receptor. His follow-up mutagenic, phenotypic, and sequencing analyses allowed him to define the cascade, which became the first quorum-sensing circuit. LuxI makes the autoinducer, and the autoinducer accumulates with increasing cell density. LuxR binds the accumulated autoinducer, the LuxR-autoinducer complex binds DNA to activate transcription of the luciferase operon, and the bacteria, as a collective, make light (Engebrecht *et al.* 1983; Engebrecht and Silverman 1984).

I was riveted! I was rather lost in how one did mutagenesis, mapped genes, and performed complementation studies, but I did think the glow-in-the-dark bacteria were stunningly beautiful and the simplicity of turning off the lights to identify which colonies glowed and which were dark appealed to me. Most importantly, I was struck by the concept that the bacteria were communicating and working as a collective. Now we know that quorum-sensing-mediated communication and group behaviors are the norm in the bacterial world, but, at the time, that was an absolutely outlandish and wholly new idea. The dogma was that bacteria were too primitive to have social and collective behaviors, rather, such sophisticated traits were the purview of eukaryotes.

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I ran up to the podium and asked Mike to hire me as his postdoc. It was in his lab that I became a geneticist (and also learned molecular biology!). Our first goal was to understand how cell–cell communication, together with other sensory inputs, controlled bioluminescence. We chose to study *Vibrio harveyi*, a bioluminescent marine bacterium closely related to *V. fischeri*. Mike had succeeded in getting genetic techniques working in *V. harveyi* but not in *V. fischeri*, where he was constrained by needing to move genes into *E. coli* to study function. We wanted to study the process of cell-cell communication *in vivo*.

I initiated my postdoc work by performing transposon mutagenesis in *V. harveyi* to identify dark mutants. My plan was to dispense with the luciferase *luxCDABE*, *luxI*, and *luxR* genes. I would then figure out what other interesting elements might be involved in cell–cell communication as assessed by the regulation of bioluminescence. Try as I might, the only transposon insertions that yielded dark mutants landed in the luciferase structural genes (*luxCDABE*). Not hitting regulatory genes was confounding because I knew there was an autoinducer, so there had to exist a synthase and a receptor. After fiddling around and failing repeatedly, I came to the realization that if there were two autoinducers and two receptors, and if they functioned in parallel, my strategy could not possibly work. With that "epiphany," I changed tactics and designed a screen to identify dim, not dark, mutants. My rationale was that such mutants must possess functional luciferase genes in order to produce some light but luciferase was mis-regulated, resulting in reduced emission. This screen, together with companion analyses, yielded genes encoding an autoinducer synthase, two two-component autoinducer receptors, and a response regulator protein that integrated the information from the two arms of the circuit (Bassler *et al.* 1993, 1994). The implication was that information flow occurred via phospho-relay. There was also clearly an additional autoinducer that I named AI-2 for "Autoinducer-2," and, therefore, also a missing autoinducer synthase gene. Collectively, the work demonstrated the existence of different kinds of cell–cell communication systems (the LuxIR-type and the two-component-type) and, perhaps most surprisingly, that bacteria could use more than a single autoinducer to communicate.

I moved to Princeton to launch my independent career. We began by using genetic screens to hunt for the mysterious AI-2 synthase. We identified a candidate gene that we named *luxS* but the gene was an orphan ORF that gave no clues to the identity of the molecule. However, with the *luxS* DNA sequence in hand, we could perform a BLAST search. To our delight, the computer showed us that *luxS* homologs existed broadly in bacterial genomes (Surette *et al.* 1999). We got our hands on a large variety of bacterial species, and we showed that they all made an AI-2 activity. By contrast, other autoinducers that we knew about from *V. harveyi* and *V. fischeri*, and by then other bacteria, appeared restricted to one, and only one, species. We put forward the idea that bacteria used the restricted autoinducer for intraspecies communication but used AI-2 for interspecies communication.

We next set out to identify the structure of the enigmatic AI-2. We purified the LuxS enzyme and determined its substrate, *S*-ribosylhomocysteine (SRH). However, the product, a 5-carbon molecule called DPD that is produced from the ribose moiety of SRH, rapidly rearranges into cyclic forms, so we were stumped as to which molecule is the active AI-2 signal molecule. Working with my Princeton colleague, structural biologist Fred Hughson, we crystallized the *V. harveyi* receptor with the active AI-2 trapped in the ligand binding site. To our amazement, we found that DPD makes an adduct with borate to produce the vibrio AI-2 (Chen *et al.* 2002). Prior to that, boron had almost no known role in biology. We learned that the marine environment is loaded with boron, making borate addition to DPD especially favorable in the ocean. We went on to show that terrestrial bacteria that employ AI-2 for quorum sensing use a differently rearranged form of DPD lacking borate, which fits with the fact that terrestrial environments are boron-limited (Miller *et al.* 2004).

Although I had fallen in love with genetics, and, at heart, I consider myself a geneticist, the AI-2 work made us realize that we could do "genetics plus." We increasingly began to incorporate biochemistry, chemistry, structure, biophysics, imaging, and engineering approaches into our experimental repertoire. This strategy allowed us to demonstrate the existence of a chemical lexicon: bacteria use specific chemical "words" to detect self (intraspecies communication), related family members (intragenera communication), others (interspecies communication), and nonbacteria (interdomain communication) (Higgins *et al.* 2007; Ismail *et al.* 2016; Papenfort *et al.* 2017; Silpe and Bassler 2019). In every case, the molecules we discovered are simple, but all were previously unknown to mankind. We also moved our studies to the human health front and began studying quorum sensing in the global pathogens *Vibrio cholerae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. In these cases, and many more, we or other groups have shown that quorum sensing controls biofilm formation and virulence (Miller *et al.* 2002; Mukherjee *et al.* 2018; Bridges and Bassler 2019). We discovered that bacteria possess strategies for interfering with each other's quorum-sensing circuits (Xavier and Bassler 2005), and, inspired by this, in an applied arm of our work, we developed synthetic quorum-sensing interference strategies that can halt infection in animal models (Swem *et al.* 2009; O'Loughlin *et al.* 2013; Ng *et al.* 2012).

We have expended much effort to learn how bacteria integrate and transduce the information encoded in autoinducer blends to drive synchronous changes in behavior. In one example of the mechanisms we uncovered, we discovered that a set of redundant small regulatory RNAs lie at the heart of vibrio quorum-sensing circuits and drive precise alterations in expression of about 600 genes. The small RNAs function post-transcriptionally, similar to microRNAs in eukaryotes. In collaboration with my Princeton colleague Ned Wingreen, a biophysics theorist, we used a computational approach to identify four small RNAs in *V. cholerae* and five small RNAs in

V. harveyi, and only by making, respectively, quadruple and quintuple null mutants was there an effect on quorum sensing (Lenz *et al.* 2004). In the ensuing years, we have found unique features and roles for each of the small RNAs; however, we still do not understand why such extreme redundancy is warranted (Feng *et al.* 2015; Rutherford *et al.* 2015).

Most recently, we discovered that eukaryotes and viruses participate in quorum-sensing-mediated conversations. We found that human intestinal epithelial cells produce an AI-2-mimic. It is possible that mammals, by exploiting the universal AI-2 activity rather than a highly species-specific autoinducer, can maximally manipulate bacterial behavior in mixed populations such as those that exist in the gut (Ismail *et al.* 2016). We showed that the human host teams up with its microbiome to use quorum sensing to defend itself against bacterial invaders. Finally, we found that the information encoded in the newest autoinducer that we discovered, called DPO, can be hijacked by a phage (Silpe and Bassler 2019). The phage encodes the DPO receptor which enables it to "eavesdrop" on bacterial quorum sensing and kill the host bacterial cells exclusively at high cell density. Presumably, this insidious strategy maximizes phage transmission to the next cell. Using what we learned, we made a set of re-engineered eavesdropping phages that respond to cues that we specify. We hope they represent new possibilities for phage therapies that will kill bacterial pathogens on demand. With these findings, we have now demonstrated that interactions across all domains of life – eukaryotic, bacterial, and viral – all depend on quorum sensing.

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