ACS chemical biology

Helen Blackwell: Deciphering the Chemical Language of Quorum Sensing

A long-standing truism in science and elsewhere is that many can accomplish more than just one. It is the ostensible reason behind the phenomenon known as quorum sensing, in which bacteria send out chemical signals to assess local population density. Once concentrations of these signals reach a certain threshold, a sign of high density, some bacterial species flip on genes that control behaviors effective only when microbes amass in a group, such as luminescence, biofilm formation, or the production of virulence factors meant to evade or overwhelm a host's immune defenses. Though the study of quorum sensing has largely been limited to bacteriologists, organic chemists, including Helen E. Blackwell, Ph.D., have recently begun using tools unique to their field to investigate the chemical language that bacteria use to communicate with each other. At her laboratory at the University of Wisconsin—Madison, Blackwell and her colleagues are taking advantage of microwave-assisted organic chemistry, solid-phase synthesis, and combinatorial chemistry to create mimics of quorum sensing molecules. Using these methods to develop libraries of small molecules and peptidomimetics and then testing them on bacterial species known to exhibit quorum sensing behavior, Blackwell's team has discovered potent antagonists and superagonists that block or stimulate quorum sensing circuits, respectively. These molecules have potential as basic research tools to investigate quorum sensing from new angles, as well as to serve as new weapons in the never-ending battle humans wage against harmful bacteria.

Longtime Chemist, Dabbling Biologist. Blackwell was born in 1972 in the Cleveland, OH, suburb of Shaker Heights to a mother who is a musician and a father who is an academic chemist. She remembers a childhood infused with both parents' interests, but by elementary school, science won out. For a time capsule that was buried on her school's grounds when Blackwell was in fourth grade, students were asked to speculate on what they wanted to do when they became adults. "I said that I wanted to be a scientist. It was one of the first times I had to say it out loud," Blackwell recalls.

As she progressed into high school, she says, her interest in science in general, and chemistry in particular, continued to grow. She enjoyed and excelled in her math and chemistry classes. "I really enjoyed the thrill of discovery, learning new things, and understanding why things were they way they were in the world around me," Blackwell says.

When it became time to choose a college, she decided to attend Oberlin College in Oberlin, OH, a school known for its sciences as well as music, an interest that Blackwell continued to enjoy and pursue through the violin. Like many schools, Oberlin requires students take "January terms," month-long semesters in which students focus on a single, self-inspired project. For her first January term, Blackwell chose to assist with graduate-level organic and polymer chemistry research at Case Western Reserve University in the laboratory of Virgil Percec, Ph.D., who is now a faculty member at the University of Pennsylvania in Philadelphia. She remembers enjoying the distinctly different feel that the higher-level

Profiles provide insights into the lives, backgrounds, career paths, and futures of scientists who serve as Experts on *ACS Chemical Biology*'s online Ask the Expert feature. Blackwell will begin answering your questions in mid-November, 2008. Readers are encouraged to submit their questions to the Experts at http://community.acs.org/chembiol/. The editors will post the most interesting exchanges on the web site.

Published online November 21, 2008
10.1021/cb800274h CCC: $40.75

research brought compared with her undergraduate science classes and laboratories. By the end of her freshman year, she had declared chemistry as a major.

Blackwell recalls the chemistry curriculum at Oberlin as a relatively grueling one; however, she notes that she was encouraged and supported by the department's dedicated professors, especially Albert Matlin, Ph.D., an organic chemist whose interests range from photochemistry to metalloenzyme mimics, and Norman Craig, Ph.D., a longtime faculty member who recently retired. "They were strong advocates for me, and they gave me a lot of guidance with my career choices. I really benefited from their mentorship," she says. She adds that she also benefited from the wealth of undergraduate research she participated in at Oberlin, including work in Matlin's laboratory, and a variety of summer industry internships, such as one at Bayer AG in Germany.

As she neared the completion of her undergraduate degree, Blackwell began investigating graduate schools. "I didn't know whether I wanted to be in academia or industry, but I knew I wanted to be in a position where I could do research and lead myself or a group to make exciting discoveries," she says. She also knew she wanted a change from the environment she'd always known. "I decided that this was the time I should take a big step away from Ohio, so I started looking at schools on the West Coast," she adds.

The California Institute of Technology (Caltech), in Pasadena, immediately appealed to her. Blackwell remembers that the school's small size and feel, similar to Oberlin's, felt like a good fit. She was also intrigued by the large number of faculty members doing work that interested her, including Robert H. Grubbs, Ph.D., a chemist whose laboratory studies organic synthesis, polymer chemistry, and organometallic chemistry, and who shared the 2005 Nobel Prize in Chemistry for developing the olefin metathesis reaction. Upon entering Caltech in the fall of 1994, Blackwell joined Grubbs' laboratory just a few months afterward. "I liked the people, and I liked the culture," says Blackwell of Grubbs' laboratory. "Bob was hands-off—he let people explore on their own, which can be good or bad because you can really excel or really flounder if you are not careful. But I liked the freedom for growth. It was pretty clear early on that this was the lab for me."

Because of her interest in polymers and her experience synthesizing these molecules during her undergraduate research, Blackwell recalls that she almost ended up with a polymer synthesis project in Grubbs' laboratory. However, then-postdoctoral fellow Scott Miller, now a member of the chemistry faculty at Yale University, encouraged her to join a project he was developing studying olefin metathesis in the context of peptidic substrates. Miller was interested in investigating whether olefin metathesis was compatible with peptides, and if so, could it be used to stitch peptides together to create novel structures. Miller got the project on excellent footing and then bequeathed it to Blackwell when his postdoctoral studies ended.

She recalls the time working with Miller as saturated with learning. "[Miller] was a fantastic mentor. My scientific life would be quite different if I hadn't joined this project," she says. Besides cementing some of the basics of being a scientist, such as designing effective experiments and writing clearly, Blackwell says she also had a crash course in biology and biochemistry, fields that had never held her interest before. Her work necessitated that she learn about peptide structure and function, a topic that led to an increasing interest of the structure and function of other biological molecules. "I'd thought before about synthetic macromolecules and how their structures influence functions such as elasticity and tensor strength, but biological molecules are macromolecules too, and their physical properties affect function in organisms. This line of thinking opened a whole new world for me," Blackwell says.

Pursuing Plants. By the time she finished her doctoral degree, Blackwell had published six papers exploring olefin metathesis (*1, 2*). She and her colleagues had found that performing this reaction on peptides is possible, creating novel structures that were constrained to adopt a range of secondary structures by introducing new carbon double bonds. She had also met her now husband, David Lynn, a fellow member of the Grubbs' laboratory. For Blackwell's postdoctoral fellowship, she sought a laboratory that would help her combine her knowledge in chemistry with her newfound interest in biology. She and Lynn also wanted to coordinate their fellowships so that they would be in or near the same city, not an easy feat for two young scientists. But they managed to work out a plan.

In 1999, Lynn headed to Robert Langer's laboratory at Massachusetts Institute of Technology, and Blackwell headed to the laboratory of Stuart L. Schreiber, Ph.D., a Harvard chemical biologist. Schreiber's main focus was chemical genetics, the idea of using small molecules to perturb protein functions in a way similar to how geneticists alter protein functions with random mutations. To accomplish this goal, Schreiber and his colleagues needed an efficient way to create vast libraries of compounds to test their ability to manipulate proteins. When she joined the laboratory, Blackwell began working on a project to develop new combinatorial and solid-phase methods to synthesize diverse molecules (*3, 4*).

She soon found herself in a learning curve even steeper than her first foray into peptide chemistry in Grubbs' laboratory. Schreiber had a large and diverse group composed of students and postdoctoral fellows in Harvard's chemistry department and medical school, so she found herself continually surrounded by a variety of researchers involved in areas previously unfa-

miliar to her, such as molecular biologists, geneticists, cell biologists, and medical doctors. However, she says that the laboratory's supportive and interactive culture helped her quickly pick up the knowledge she needed to work on her project. "It's like learning a language," she recalls. "You immerse yourself in it, you pick up key words, and at the end you find you're talking in that language."

Eventually, Blackwell became interested in moving her project in a direction that Schreiber's laboratory had never explored. Though other researchers in the laboratory were using chemical genetic techniques on a range of model organisms, from yeast to zebrafish, none were exploring this method in plants. Plants are uniquely suited to a chemical genetics approach, explains Blackwell, because of their large genomes containing many replicated genes. Traditional genetic techniques can be challenging in plants because knocking out a function might involve locating and blocking the functions of numerous genes. However, using a molecule to perturb the protein responsible for a function could theoretically be the equivalent of knocking out an entire gene family.

Schreiber gave Blackwell his support on the project, and as extra encouragement, Lynn gave her some *Arabidopsis thaliana* seeds for her birthday that year. "People would tell me, wow, you're straying a long way from where you started," Blackwell says, describing her journey from polymer chemistry to olefin metathesis to plant genetics. "But one of the best parts of being in academics is that you can, theoretically, take your research where you want to go. And this is where I wanted to go," she adds. Over the next year, she and her colleagues used chemical genetics to develop novel assays that netted new *Arabidopsis* phenotypes, publishing several papers on the topic (*5, 6*).

Toward the end of her postdoctoral fellowship, Blackwell and Lynn began applying to research institutions across the country. Any that had positions open in each of their departments of interest—engineering for Lynn and chemistry for Blackwell—was fair game, she says. In 2002, they were both offered positions at the University of Wisconsin—Madison, which is where Blackwell and Lynn work today.

Gathering a Laboratory Quorum. Starting her new laboratory was "an overwhelming process, as it is for any new faculty member," Blackwell recalls. In the daunting jumble of recruiting students, teaching for the first time, and hunting for funding sources, she took solace in exploring ideas for research projects that would define the scope of her laboratory. Initially, she and her colleagues began developing new microwave-assisted reactions to improve the speed of synthesizing small organic molecules on solid supports (*7, 8*). Her laboratory also began developing a class of peptidomimetics called peptoids, oligomers of N-substituted glycine (*9, 10*) and developing screens to test these molecules for potentially interesting biological functions (*11*).

Blackwell was also interested in continuing work on chemical signaling in plants to explore what molecules control their wide range of tropisms, including the ability to respond to gravity and light, and to search for compounds that might block these basic responses (*12*). However, as she learned more about what was already known about these systems, she realized that the research was still in a very early stage—little had been discovered about these responses on a molecular level, giving Blackwell no defined knowledge about critical targets to block or mechanisms to disrupt. "You can take a chemical approach and try to tease these mechanisms apart, but there are a lot of risks associated with that and it could be painstakingly slow," says Blackwell. "I wanted to lean more toward hypothesis-focused research at the outset."

In the course of reading up on plant signaling methods, Blackwell says she became intrigued by how certain species of plants respond to bacterial invasion, a phenomenon typical in legumes, which often rely on a symbiotic relationship with nitrogen-fixing bacteria. The plants and microbes appear to engage in chemical signaling cross-talk that allows them to recognize each other and set up a relationship. Blackwell eventually became interested in other research focusing just on the chemical signals, or autoinducers, that bacteria rely on for quorum sensing. Though understanding plants' chemical signals had seemed like a daunting challenge, investigating bacterial autoinducers was more tractable, Blackwell says. "As I learned more and more, I thought this would be a great place to go with the chemical methods and screens we were developing and how the lab was evolving. It really shaped where we are today."

Although the work might appear to have a purely biological focus, she adds, investigating quorum sensing is also well-suited for chemistry. "What's fascinating to me as a chemist is that this phenomenon is completely based on chemistry. The fundamental nature of quorum sensing is small molecules mediating gene transcription. Without chemical signals, bacteria can't perform any of the group behaviors that they rely on," she says. Quorum sensing work also has a very practical use, not just for basic microbial research but also in medicine for treating infections, Blackwell explains. If chemists could develop molecules to inhibit quorum sensing or perhaps selectively incite quorum sensing behavior when population density is low, it might be possible to prevent bacterial mobs from becoming virulent and make infections easier to treat with traditional antibiotics.

By her second year at the University of Wisconsin, her laboratory's fresh focus on quorum sensing began to take off. Using solid-phase, microwave-assisted reactions combined with combinatorial methods, Blackwell and her colleagues began developing a library of *N*-acyl-L-homoserine lac-

tones, compounds bacteria use in quorum sensing, to test their activity on bacterial species known to exhibit this behavior. In an initial milestone, she and her laboratory tested a small library of ~30 ligands, including copies of natural autoinducers and synthetic variants, on *Agrobacterium tumefaciens*, a common soil bacterium, and *Pseudomonas aeruginosa*, an opportunistic pathogen that frequently infects the lungs of cystic fibrosis patients (*13*). The researchers reported that even in such a small chemical library, they were able to locate synthetic autoinducers that inhibited quorum sensing at the same level or more potently than many other compounds reported previously.

Taking their research an additional step, Blackwell and her team developed another small library of *N*-phenylacetanoyl-L-homoserine lactones, testing it this time on *Vibrio fischeri*, a well-studied symbiotic bacterium that lives in the light organs of squids and luminesces when it reaches a high population density, an activity that aids the squids in hunting (*14*). The researchers found that this library contained not only potent antagonists that inhibited *Vibrio*'s quorum sensing circuits but also a powerful superagonist that effectively encouraged quorum sensing to take place. Blackwell's laboratory is now collaborating on this project with Edward Ruby, a University of Wisconsin microbiologist who is one of the world's experts on this symbiotic relationship and raises the squids in his laboratory. Preliminary data show that these compounds are also active *in vivo*.

Most recently, Blackwell's laboratory extended their quorum sensing research in a new and far more challenging direction. Rather than looking at just one bacterium in isolation, Blackwell says that she and her colleagues are interested in studying bacteria in a more natural environment that includes multiple species. "Working with just one species is hard enough, but when they're mixed together, the experimental techniques grow exponentially. But I think such complex systems are where we need to be headed," she says.

In a step toward that end, Blackwell and her colleagues recently reported the results of comparatively testing another library of nearly 100 compounds on three species of bacteria, *A. tumefaciens*, *P. aeruginosa*, and *V. fischeri*, to determine whether any of the compounds could affect quorum sensing in similar or contrasting ways in the three organisms (*15, 16*). The researchers found several compounds that affected quorum sensing in just one of the species. However, some of the compounds had activity in at least two species, suggesting that both species-specific and broad spectrum quorum sensing modulators can be developed. She and her team are particularly excited about using such selective ligands in mixed microbial systems that are pervasive in the environment, such as in soil and aquatic settings. Understanding which chemical signals target individuals or overlap between species could be a useful tool in medicine, Blackwell explains, either helping doctors keep the side effects of a treatment low by targeting a single type of microbe while avoiding harm to helpful bacteria or by acting as a broad ranging anti-infective agent to wipe out several harmful species at once. Finding these useful compounds in what's still considered a small library of 100 compounds "tells us that we've just scratched the surface of the known non-native chemical modulators of quorum sensing," Blackwell says (*17*).

Giving Back, Keeping Chemistry. In the future, Blackwell plans to continue developing new compounds, including small molecules and peptidomimetics, to test on quorum sensing behavior, moving her experiments into progressively more complex multispecies bacterial communities in hosts and the environment. She also plans to continue the range of other projects her laboratory is pursuing, including testing their compounds for traditional antibacterial activity. Using small-molecule macroarrays, she and her colleagues have already discovered several promising agents against *Staphylococcus aureus*, an organism that is currently one of the most common species blamed in antibiotic-resistant infections (*18*). "There is still an urgent need for antibacterial agents that work through traditional mechanisms," Blackwell says.

Besides time spent in the laboratory with students, Blackwell also carries a substantial teaching load, part of her job that she says she's taken "very seriously" during her time at the University of Wisconsin. Although she enjoys working hands-on with students in the laboratory, she explains that she also deeply enjoys teaching in the classroom, especially undergraduate organic chemistry courses. "These are the classes many undergraduates hate, as they believe it's the hardest class they'll ever take in their lives," she says. "I try to show them that it's nowhere near as bad as they think it will be, indeed, that it's relevant to almost everything in their daily lives and that many people actually grow to like organic chemistry. Some people like it so much that they go on to become organic chemistry professors," she laughs.

Blackwell also works with undergraduates in her own laboratory, an opportunity that she considers a critical way to pay forward the opportunities she received. "As an undergrad, if I hadn't had experience in a graduate group, I would not be where I am now. My life would have been very different if I hadn't become involved with research early and seen what it is about. Giving undergraduates that opportunity is very important, and I'm committed to giving back that way," she says. She adds that working with budding young researchers is also helpful for her graduate students and postdocs, who benefit from gaining mentoring experience.

Eventually, Blackwell hopes to grow her laboratory beyond the current 10 graduate students and one postdoctoral fellow to pick

up more students and fellows with knowledge of bacteriology, an area of ever-increasing interest as her work on quorum sensing develops. During an upcoming sabbatical, she plans to use a heavy portion of the time learning more hands-on bacteriology techniques to aid her research. But though she's hoping to grow the bacteriology side of her work, she says that her laboratory's focus will never stray from chemistry. "We'll never lose chemistry because we need that ability to make these molecules and improve them and understand their mechanism of action," she says. "We'll need more biology to move to the next level, but we'll always approach our research problems through the lens of chemistry. Chemistry is our foundation."

—Christen Brownlee, Science Writer

REFERENCES

1. Blackwell, H. E., O'Leary, D. J., Chatterjee, A. K., Washenfelder, R. A., Bussmann, D. A., and Grubbs, R. H. (2000) New approaches to olefin cross-metathesis, *J. Am. Chem. Soc. 122*, 58–71.
2. Blackwell, H. E., and Grubbs, R. H. (1998) Highly efficient synthesis of covalently cross-linked peptide helices by ring-closing metathesis, *Angew. Chem., Int. Ed. 37*, 3281–3284.
3. Blackwell, H. E., Pérez, L., Stavenger, R. A, Tallarico, J. A., Cope, Eatough, E., Foley, M. A., and Schreiber, S. L. (2001) A one-bead, one-stock solution approach to chemical genetics: part 1, *Chem. Biol. 8*, 1167–1182.
4. Blackwell, H. E., Pérez, L., and Schreiber, S. L. (2001) Decoding products of diversity pathways from stock solutions derived from single polymeric macrobeads, *Angew. Chem., Int. Ed. 40*, 3421–3425.
5. Zhao, Y., Dai, X., Blackwell, H. E., Schreiber, S. L., and Chory, J. (2003) SIR1, an upstream component in auxin signaling identified by chemical genetics, *Science 301*, 1107–1110.
6. Grozinger, C. M., Chao, E. D., Blackwell, H. E., Moazed, D., and Schreiber, S. L. (2001) Identification of a class of small molecule inhibitors of the sirtuin family of NAD-dependent deacetylases by phenotypic screening, *J. Biol. Chem. 276*, 38837–38843.
7. Bowman, M. D., Jeske, R. C., and Blackwell, H. E. (2004) Microwave-accelerated SPOT-synthesis on cellulose supports, *Org. Lett. 6*, 2019–2022.
8. Blackwell, H. E. (2006) Hitting the SPOT: small-molecule macroarrays advance combinatorial synthesis, *Curr. Opin. Chem. Biol. 10*, 203–212.
9. Gorske, B. C., and Blackwell, H. E. (2006) Tuning peptoid secondary structure with pentafluoroaromatic functionality: a new design paradigm for the construction of discretely folded peptoid structures, *J. Am. Chem. Soc. 128*, 14378–14387.
10. Gorske, B. C., Bastian, B. L., Geske, G. D., and Blackwell, H. E. (2007) Local and tunable n→π* interactions regulate amide isomerism in the peptoid backbone, *J. Am. Chem. Soc. 129*, 8928–8929.
11. Gorske, B. C., and Blackwell, H. E. (2006) Interception of quorum sensing in *Staphylococcus aureus*: a new niche for peptidomimetics, *Org. Biomol. Chem. 4*, 1441–1445.
12. Blackwell, H. E., and Zhao, Y. (2003) Chemical genetic approaches to plant biology, *Plant Physiol. 133*, 448–455.
13. Geske, G. D., Wezeman, R. J., Siegel, A. P., and Blackwell, H. E. (2005) Small molecule inhibitors of bacterial quorum sensing and biofilm formation, *J. Am. Chem. Soc. 127*, 12762–12763.
14. Geske, G. D., O'Neill, J. C., and Blackwell, H. E. (2007) *N*-Phenylacetanoyl-L-homoserine lactones can strongly antagonize or superagonize quorum sensing in *Vibrio fischeri*, *ACS Chem. Biol. 2*, 315–319.
15. Geske, G. D., O'Neill, J. C., Miller, D. M., Mattmann, M. E., and Blackwell, H. E. (2007) Modulation of bacterial quorum sensing with synthetic ligands: systematic evaluation of N-acylated homoserine lactones in multiple species and new insights into their mechanisms of action, *J. Am. Chem. Soc. 129*, 13613–13625.
16. Geske, G. D., O'Neill, J. C., Miller, D. M., Wezeman, R. J., Mattmann, M. E., Lin, Q., and Blackwell, H. E. (2008) Comparative analyses of N-acylated homoserine lactones reveal unique structural features that dictate their ability to activate or inhibit quorum sensing, *ChemBioChem 9*, 389–400.
17. Geske, G. D., O'Neill, J. C., and Blackwell, H. E. (2008) Expanding dialogues: from natural autoinducers to non-natural analogues that modulate quorum sensing in Gram-negative bacteria, *Chem. Soc. Rev. 37*, 1432–1447.
18. Bowman, M. D., O'Neill, J. C., Stringer, J. R., and Blackwell, H. E. (2007) Rapid identification of antibacterial agents effective against *Staphylococcus aureus* using small-molecule macroarrays, *Chem. Biol. 14*, 351–357.