Point of VIEW

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Learning the Language of Bacteria

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ABSTRACT Bacteria "talk" with each other by using small molecules that enable individuals in a population to coordinate their behavior. This language is termed quorum sensing. Bacterial pathogens may use this language to decide when to attack a host organism; therefore, the development of artificial signals to interfere with this signal process has become an area of intense chemical research.

A remarkable change to the classical view of the microbial world has become apparent over the last few decades. Rather than bacteria being seen as purely single-celled organisms, existing in isolation, it has become clear that bacteria mainly crowd together in highly complex, multispecies communities. Moreover, they actually "talk" to one another by using small molecules. In Gram-negative bacteria, these small molecules are commonly N-acylated homoserine lactones (AHLs). The design and synthesis of AHL analogues have been performed by many groups (*1*–*8*) on a more or less *ad hoc* basis, making it difficult to draw any firm conclusions about structure–activity relationships and species selectivity. Blackwell and co-workers (*9*) now report a comprehensive and systematic study that compares the activities of ~100 AHL analogues across three species. This not only has unveiled a new insight into the structural similarities and differences of the AHL receptor pockets but also has identified some of the most potent synthetic modulators of quorum sensing. Moreover, this study has revealed that certain analogues display broad-spectrum activity, whereas others show marked species selectivity, thus delivering a valuable set of chemical tools to probe quorum sensing signaling.

AHL molecules are used for quorum sensing in many important animal and plant pathogens (*10*–*16*), including *Pseudomonas* sp. and *Agrobacterium tumefaciens*, as well as nonpathogenic organisms like the marine bacterium *Vibrio fischeri*. In fact, the phenomenon of quorum sensing was first recognized >30 years ago in *V. fischeri* (*17*, *18*), although the significance of the observations took time to become widely recognized. *V. fischeri* is a symbiont that colonizes the light-producing organ of certain marine fish and squid. The host supplies the colonized bacteria with nutrients so that they thrive to exceed a population cell density of 10^{10} cells mL^{-1}. When this population threshold is exceeded, transcription of the gene cluster encoding the light-producing machinery (the *luxCDABEG* operon) is activated. The term "quorum sensing" was coined to describe this behavior, because the population must reach a "quorum" before any light is produced (*19*). *V. fischeri* uses the small molecule *N*-(3-oxohexanoyl)-L-homoserine lactone (OHHL) to measure population density (Figure 1). Each cell within the population uses the enzyme LuxI to produce a continuous low level of freely diffusible OHHL. Although the amount of OHHL produced by individual cells is low, the population *en masse* accumulates it to high concentrations. When the concentration of OHHL is high enough, it binds to a cytoplasmic receptor known as LuxR, which is a transcriptional regulator. This causes LuxR to dimerize and adopt the active conformation, leading to transcription of the *luxCDABEG* cluster. The LuxR—OHHL complex also activates the expression of *luxI* further, generating a positive feedback loop. This behavior is known as autoinduction, and AHL derivatives are often known as autoinducers.

A. tumefaciens is a plant pathogen that has its quorum sensing system encoded on a plasmid, rather than the bacterial chromosome. The tumor-inducing plasmid is

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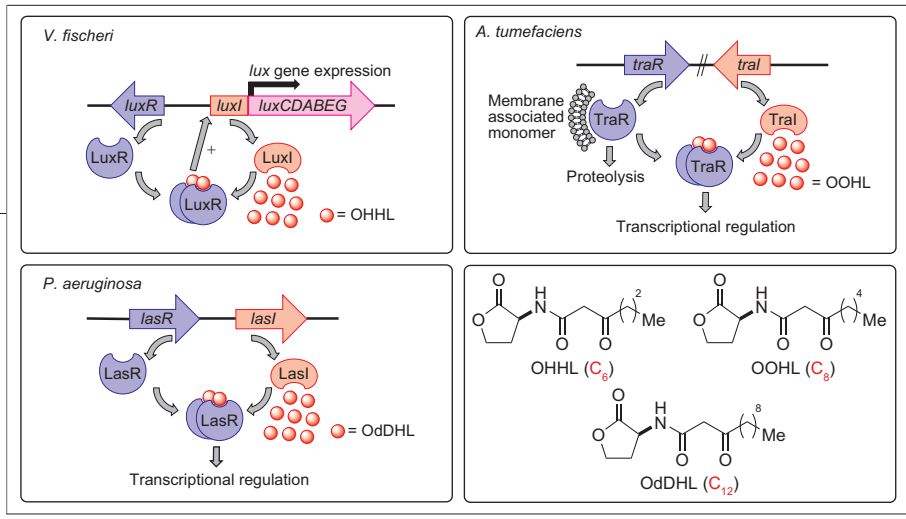

Figure 1. Schematic of the quorum sensing systems of *V. fischeri*, *A. tumefaciens*, and *P. aeruginosa* (only the *las* system is shown), including the structures of natural AHL molecules that are used by these bacteria. The number of carbons (C_n) in the acyl chain is indicated for clarity.

transferred directly into the nucleus of host plant cells, where it stimulates overproduction of growth hormones and consequent tissue proliferation to generate a crown gall tumor. The plasmid encodes a LuxI-type protein called TraI that makes *N*-(3-oxooctanoyl)-L-homoserine lactone) (OOHL), which is the cognate AHL recognized by the LuxR homologue TraR (Figure 1). In the apo state, TraR is a membrane-associated monomer, which is unstable in the absence of the ligand and is degraded rapidly by endogenous proteases. This protein turnover is prevented in the presence of bound OOHL, and the stable dimeric complex is a transcriptional activator.

P. aeruginosa has one of the most complicated quorum sensing systems, yet it is one of the best characterized because of the clinical importance of this species. *P. aeruginosa* has been associated with ~10% of all nosocomial (hospital-acquired) infections, and it is the main contributor to progressive lung degeneration in many cystic fibrosis patients (*20*). Quorum sensing in *P. aeruginosa* involves two discrete AHL molecules (OdDHL and BHL) that are generated and sensed by two separate signaling systems (LasIR and RhlIR, respectively). Together, these signaling systems control production of a diverse variety of virulence factors and Biofilm production. The *rhl* system is subordinate to the *las* system; therefore, the LasR receptor is usually the main focus for inhibitor development and biochemical studies. Similar to TraR, LasR requires its cognate ligand for folding and dimerization into its transcriptionally active state.

What Blackwell and co-workers have so elegantly undertaken is a comprehensive structure–activity relationship study across these three important bacterial species (*9*). Because past work has tended to focus on just one organism at a time (often using different reporter strains and assay procedures), or has looked at a limited subset of AHL structural analogues, it is difficult to use the results for the design of new ligands or to predict species selectivity. To directly address these issues, the Blackwell group made a wide range of AHL analogues by using an efficient solid-supported, microwave-assisted synthetic route. It is important to recognize this chemical contribution to the study. The high-throughput chemistry and chemical expertise enabled the high-throughput screening. A striking illustration of a major conclusion from the study is shown in the Venn diagrams shown in Figure 2. This highlights the fact that both broad spectrum and species-selective antagonists are possible and have now been identified. In contrast, agonism of each LuxR-type receptor appears to be pickier. It seems like the longer the tail of the natural ligand, the more space within the binding pocket to accommodate xenobiotics; however, a larger pocket does not make it any easier to agonize the receptor. Further dose–response experiments showed that the antagonists are better described as partial agonists, because at high

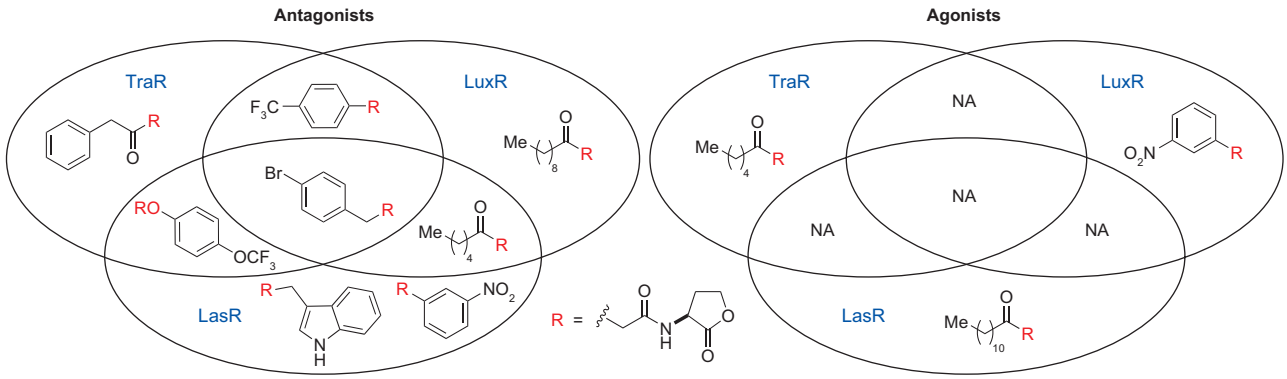

Figure 2. Venn diagrams illustrating potent agonists and antagonists and their selectivity for the different LuxR homologues from *V. fischeri* (LuxR), *A. tumefaciens* (TraR), and *P. aeruginosa* (LasR). NA = no applicable ligands identified.

concentrations they were able to activate the transcriptional regulators. All the analogues tested have the homoserine headgroup; nevertheless, it is probably not the case that all the molecules are binding in exactly the same way as the natural ligand. Docking studies support this hunch (*9*). This binding flexibility makes it very difficult to undertake structure-based design without continual structural information on analogues.

The discovery of broad-spectrum and species-selective AHL analogues should be useful in chemical biology experiments aimed at exploring the importance of quorum sensing in a wide range of bacterial processes, such as virulence, Biofilm formation, and interspecies interactions. Real-life applications (such as a chemotherapeutics, antifouling coatings, or additives in antibiotics or even toothpaste) seem a long way off; however, a synthetic derivative of a natural product from marine algae has generated a lot of excitement recently (*21*). This has been used to attenuate *P. aeruginosa* virulence in a lung infection mouse model.

3-Oxo-AHL compounds have the potential to be converted to tetramic acids *in vivo* (*22*). The tetramic acid from OdDHL has been shown to have antibacterial properties, especially against Gram-positive strains. Bacteria such as *P. aeruginosa* may use this as a way to prevent competing bacteria from occupying the same environment. The tetramic acid is also an excellent siderophore. It would be interesting to see whether any derivatives from the Blackwell compounds had similar or even enhanced effects.

Fundamental research into quorum sensing has revealed unpredictable discoveries. For instance, some AHL molecules have effects on other species, including the host organism. For example, OdDHL has been shown to cause inflammation and modulate an immune response (*23*). Several analogues have shown similar effects (*24*). The basis of these observations remains to be uncovered and may reveal new drug target and small molecule leads. What is clear is that the quorum sensing field is a fertile one for chemical biology. Whether this language is a good target for chemical intervention in a variety of applications remains to be seen; however, no doubt many more unexpected and exciting results will be discovered along the way.

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REFERENCES

1. Kline, T., Bowman, J., Iglewski, B. H., de Kievit, T. R., Kakai, Y., and Passador, L. (1999) Novel synthetic analogs of the *Pseudomonas* autoinducer, *Bioorg. Med. Chem. Lett. 9*, 3447–3452.
2. Frezza, M., Casting, S., Estephane, J., Soulere, L., Deshayes, C., Chantegrel, B., Nasser, W., Queneau, Y., Reverchon, S., and Doutheau, A. (2006) Synthesis and biological evaluation of homoserine lactone derived ureas as antagonists of bacterial quorum sensing, *Bioorg. Med. Chem. 14*, 4781–4791.
3. Persson, T., Hansen, T. H., Rasmussen, T. B., Skinderso, M. E., Givskov, M., and Nielsen, J. (2005) Rational design and synthesis of new quorum-sensing inhibitors derived from acylated homoserine lactones and natural products from garlic, *Org. Biomol. Chem. 3*, 253–262.
4. Jog, G. J., Igarashi, J., and Suga, H. (2006) Stereoisomers of P.*aeruginosa*autoinducer analog to probe the regulator binding site, *Chem. Biol. 13*, 123–128.
5. Glansdorp, F. G., Thomas, G. L., Lee, J. K., Dutton, J. M., Salmond, G. P. C., Welch, M., and Spring, D. R. (2004) Synthesis and stability of small molecule probes for *Pseudomonas aeruginosa* quorum sensing modulation, *Org. Biomol. Chem. 2*, 3329–3336.
6. Muh, U., Schuster, M., Heim, R., Singh, A., Olson, E. R., and Greenberg, E. P. (2006) Novel *Pseudomonas aeruginosa* quorum-sensing inhibitors identified in an ultra-high-throughput screen, *Antimicrob. Agents Chemother. 50*, 3674–3679.
7. 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.
8. 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*, 315–320.
9. 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.
10. de Kievit, T. R., and Iglewski, B. H. (2000) Bacterial quorum sensing in pathogenic relationships, *Infect. Immun. 68*, 4839–4849.
11. Whitehead, N. A., Barnard, A. M., Slater, H., Simpson, N. J., and Salmond, G. P. C. (2001) Quorum-sensing in Gram-negative bacteria, *FEMS Microbiol. Rev. 25*, 365–404.
12. Fuqua, C., and Greenberg, E. P. (2002) Listening in on bacteria: acyl-homoserine lactone signalling, *Nat. Rev. Mol. Cell Biol. 3*, 685–695.
13. Hall-Stoodley, L., Costerton, J. W., and Stoodley, P. (2004) Bacterial biofilms: from the natural environment to infectious diseases, *Nat. Rev. Microbiol. 2*, 95–108.
14. Waters, C. M., and Bassler, B. L. (2005) Quorum sensing: cell-to-cell communication in bacteria, *Annu. Rev. Cell Dev. Biol. 21*, 319–346.
15. Welch, M., Mikkelsen, H., Swatton, J. E., Smith, D. S., Thomas, G. L., Glansdorp, F. G., and Spring, D. R. (2005) Cell-cell communication in Gram-negative bacteria, *Mol. Biosyst. 1*, 196–202.
16. Smith, D., Wang, J.-H., Swatton, J. E., Davenport, P., Price, B., Mikkelsen, H., Stickland, H., Nishikawa, K., Gardiol, N., Spring, D. R., and Welch, M. (2006) Variations on a theme: diverseN-acyl homoserine lactone-mediated quorum sensing mechanisms in Gram-negative bacteria, *Sci. Prog. 89*, 167–211.
17. Nealson, K. H., Platt, T., and Hastings, J. W. (1970) Cellular control of the synthesis and activity of the bacterial luminescent system, *J. Bacteriol. 104*, 313–322.
18. Eberhard, A. (1972) Inhibition and activation of bacterial luciferase synthesis, *J. Bacteriol. 109*, 1101–1105.
19. Fuqua, W. C., Winans, S. C., and Greenberg, E. P. (1994) Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators, *J. Bacteriol. 176*, 269–275.
20. Van Delden, C., and Iglewski, B. H. (1998) Cell-to-cell signalling and *Pseudomonas aeruginosa* infections, *Emerging Infect. Dis. 4*, 551–560.
21. Wu, H., Song, Z., Hentzer, M., Andersen, J. B., Molin, S., Givskov, M., and Hoiby, N. (2004) Synthetic furanones inhibit quorum-sensing and enhance bacterial clearance in *Pseudomonas aeruginosa* lung infection in mice, *J. Antimicrob. Chemother. 53*, 1054–1061.
22. Kaufmann, G. F., Sartorio, R., Lee, S. H., Rogers, C. J., Meijler, M. M., Moss, J. A., Clapham, B., Brogan, A. P., Dickerson, T. J., and Janda, K. D. (2005) Revisiting quorum sensing: discovery of additional chemical and biological functions for 3-oxo-*N*-acyl-homoserine lactones, *Proc. Natl. Acad. Sci. U.S.A. 102*, 309–314.
23. Pritchard, D. I. (2006) Immune modulation by *Pseudomonas aeruginosa* quorum-sensing signal molecules, *Int. J. Med. Microbiol. 296*, 111–116.
24. Thomas, G. L., Böhner, C. M., Williams, H. E., Walsh, C. M., Ladlow, M., Welch, M., Bryant, C. E., and Spring, D. R. (2006) Immunomodulatory effects of *Pseudomonas aeruginosa* quorum sensing small molecule probes on mammalian macrophages, *Mol. Biosyst. 2*, 132–137.