GUEST COMMENTARY

Quorum Quenching in *Agrobacterium tumefaciens*: Chance or Necessity?[▽]

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Cell-cell communication or "quorum sensing" (QS) between members of a population is an established phenomenon that has been described for many different bacterial species. A number of different types of QS systems have been discovered; however, a unifying theme is the synthesis of a small signal molecule, often called an autoinducer or pheromone, which activates a specific response when it accumulates to a threshold concentration within a population. A relatively new and exciting aspect of the field of QS that has received much recent attention is "quorum quenching" (QQ), or interference of a QS signaling system. This occurs through either the inhibition of a QS component or the depletion of the signal itself, resulting in an attenuation of the response. In the plant pathogen *Agrobacterium tumefaciens*, an enzyme (BlcC) that destroys the bacterium's QS signal has been recently described, prompting much speculation that this enzyme is specifically involved in the quenching of the QS system. A variety of explanations for the adaptive significance of QQ in the QS system of *A. tumefaciens* and implications for the bacterium's role as a plant pathogen have been suggested in the literature (for example, see references 4 and 27). However, the role of BlcC in QQ was never directly addressed. In *A. tumefaciens*, the QS system regulates Ti (tumor-inducing) plasmid conjugation. In this issue, Khan and Farrand (12) directly address the biological significance of BlcC by examining its effect on Ti plasmid conjugation both in culture and in planta. Their study has implications for our understanding of the possible roles in *Agrobacterium* and other bacteria of BlcC-like enzymes, which are generally thought to function as quorum quenchers of proteobacteria.

QUORUM SENSING AND VIRULENCE IN *AGROBACTERIUM TUMEFACIENS*

The most widespread and best-studied type of QS system in proteobacteria is the LuxR-LuxI-type system. The LuxI-type protein synthesizes a small diffusible signal molecule called an *N*-acylhomoserine lactone (AHL), while LuxR is the cytoplasmic receptor for that signal, regulating target genes in response to inducing concentrations of the cognate AHL (26). In *A. tumefaciens*, the LuxI-type protein, called TraI, synthesizes the AHL *N*-3-oxooctanoyl-L-homoserine lactone (OOHL), which is recognized with high specificity by the receptor protein TraR.

TraI, TraR, and all known QS-regulated genes in *A. tumefaciens* occur on the Ti plasmid, which is required for the formation of tumors, called crown galls, on a wide range of host plants (25). During the infection process, a segment of the Ti plasmid is transferred to the nucleus of host plant cells, where it directs the overproduction of phytohormones (hence, the formation of a tumor) and the production of novel compounds called opines. The infecting strain of *A. tumefaciens* carries the complement of genes, again on the Ti plasmid, that are required for the utilization of the opines as sources of carbon and nitrogen. By thus harnessing the metabolism of the host plant to produce a novel food source, the bacteria provide a specialized niche for themselves and, presumably, a competitive advantage over other plant-colonizing bacteria.

The virulence system and the TraR-TraI system of the Ti plasmid are intimately linked (9, 14, 21). The expression of *traR* requires the presence of opines, which are found only at the site of a tumor. Therefore, the QS system functions only in host plants and only after infection has occurred. As active TraR-OOHL complexes are required for Ti plasmid conjugation, this means that Ti plasmid transfer is restricted to members of an infecting population on a transformed host plant. In addition, TraR-OOHL also upregulates the expression of the Ti plasmid replication genes, resulting in an increase in copy number per cell in response to QS (14, 21). This leads to the intriguing question of the QS system's role in pathogenesis. One possibility is that the increase in Ti plasmid copy number may benefit a plant-associated population through increasing the gene dose of virulence and opine utilization genes.

IS QUORUM QUENCHING IN *A. TUMEFACIENS* REAL?

The speculation that QQ may be involved in the regulation of TraR-TraI activity was prompted by the discovery of a family of lactonases that possess activity against homoserine lactones (HSLs). The first member of this family to be described, AiiA (autoinducer inactivation gene A) from *Bacillus cereus*, was shown to have a high level of activity against AHLs, hydrolyzing the lactone ring and thus inactivating signal activity (7). The expression of *aiiA* in the plant pathogen *Erwinia carotovora* (in which a LuxR-LuxI system regulates virulence factors) attenuates disease, and the same effect can be achieved by *aiiA* expression in the plant host (7). These discoveries led to the concept of QQ, and many other examples and additional mechanisms have since been reported.

The *blcC* (formerly *attM*) gene of *A. tumefaciens* is in the

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aiiA family and has been shown by three independent groups to have lactonase activity against AHLs including OOHL (2, 3, 29). One group suggested that *blcC* is induced at a high population density, which would be consistent with a role in QQ (28, 29), while in other studies, a population density effect was not observed (1, 12). The *blcC* gene is part of a three-gene operon (*blcABC*), which, since the earlier reports, has been shown to have activity against γ-butyrolactone (GBL) and in fact confers the ability to grow on GBL as the sole carbon source (1, 3). While the intermediates of GBL catabolism via BlcC and BlcB (γ-hydroxybutarate and succinic semialdehyde [SSA], respectively) are strong inducers of the operon (1, 3, 12), all attempts to demonstrate an activation of *blcC* reporter fusions with exogenous HSLs have failed (1, 3, 29). OOHL depletion via *blcC* can be achieved only by the constitutive expression (deletion of the repressor BlcR) or induction of the operon by the addition of GBL or its intermediates to the growth medium (1, 3, 12, 29). These data strongly suggest that, in fact, GBL is the substrate for which this catabolic operon was selected, and the activity of BlcC on OOHL may be coincidental. Furthermore, while BlcABC are capable of converting GBL to succinic acid (a tricarboxylic acid cycle intermediate), the ring-opened metabolite of OOHL is not further metabolized (3).

On the other hand, BlcC could be involved in the QQ of OOHL if both *blcABC* and the QS system are expressed at the same time in planta. A recent report in support of this argument showed that γ-aminobutyric acid (GABA) induces *blcABC* but indirectly through its metabolite SSA (4). This is significant since GABA is known to accumulate to high levels at wound sites in plants, which also happen to secrete inducers of *A. tumefaciens* virulence and are therefore preferred sites of infection (4, 23). However, this still does not indicate that *blcC* and the QS system are expressed simultaneously in planta with an observable QQ effect.

Khan and Farrand (12) addressed the possibility of QQ by directly examining the effect of *blcABC* on Ti plasmid conjugation. As in previous studies, they showed that *blcC* induction has an observable effect on OOHL accumulation in culture. However, in spot plate mating assays, donors that were cultured in the presence of SSA transferred the Ti plasmid at the same frequency as that of donors where *blcABC* was not induced. In these experiments, donors at different stages of growth were spotted onto a lawn of recipients on medium that is selective for transconjugants. Therefore, transient or growth-stage effects on conjugation frequency could be observed, although none were found in the above-described experiment. During infection at a wound site, one could predict that the agrobacteria would be exposed to *blc* inducers such as GABA before the opine concentration and population density reach levels that are high enough for the induction and activation of TraR. Those authors demonstrated that the preincubation of the donors with SSA before the induction of *traR* (in this case, under the control of an isopropyl-β-D-thiogalactopyranoside [IPTG]-inducible promoter) did result in an early and transient decrease in Ti plasmid conjugation frequency. Presumably, BlcC activity delays the accumulation of inducing levels of OOHL.

What about in planta? To answer this question, those authors inoculated a mix of donors (either the wild type or *blcC*

mutants) and recipients at wounds produced on the stems of tomato plants. At regular intervals postinfection, macerates of the infected plant tissue were plated onto selective medium to count donors, recipients, and transconjugants. Although an early and transient effect was observed, it disappeared by about 4 weeks postinfection (tumors are visible between 2 and 3 weeks), at which point conjugation frequency was indistinguishable whether *blcC* was expressed or not. Although BlcC does appear to be expressed in planta, it does not seem to have a lasting effect or significant impact on the biological role of TraR-OOHL in the induction of Ti plasmid conjugation (12).

Is the early and transient effect an "accident," as those authors suggested? Prior to that study, the most compelling argument that *blcC* is not involved in QQ is that it is not upregulated by AHLs and is part of an operon that confers the ability to grow on GBL but not AHLs. This also argues against a specific role of BlcC in QQ of signals from other bacteria in the rhizosphere. It was shown previously that purified AiiA has a much higher level of activity on AHLs than on GBL, and thus, its role in QQ is much more convincing (24). It is unfortunate that this direct comparison of substrate specificity has not been reported for BlcC, although we predict that activity would be at least similar if not higher for GBL than for HSLs. It is most likely that BlcC has been selected for the degradation of plant-released compounds such as GBL, and activity against HSLs may indeed be an "accident" with an effect minimal enough that it has not been selected against.

QUORUM QUENCHING IN OTHER SYSTEMS

Although self-regulation through signal depletion by BlcC is quite unlikely in *A. tumefaciens*, there are examples of bona fide quorum quenchers that function in interspecies interactions. The AiiA protein of *Bacillus* species, which can function in the competitive inhibition of QS in proteobacteria (8), is just one example of the many types of signal-depleting enzymes, including a family of AHL aminoacylases. One of the best-characterized aminoacylases is from the soil bacterium *Variovorax paradoxus*, which can use AHLs as a sole source of carbon and nitrogen (13). However, whether or not this also confers an advantage specifically through QQ of neighboring bacteria is not known. Many other soil bacteria are likely to degrade AHLs, which are in fact quite abundant in soil (5, 15). In addition to signal depletion, signaling can also be blocked at the level of the signal synthase or receptor. An excellent example of this is QS interference between different groups of the pathogenic bacterium *Staphylococcus aureus*. Like many gram-positive bacteria, *S. aureus* uses short oligopeptides as a QS signal, in this case, to induce the expression of virulence genes. In *S. aureus*, these signals (called AIPs) vary slightly in sequence between different strains, and it has been shown that the signal of one strain can block the signal receptor of another, perhaps conferring a competitive advantage during host colonization (16, 19).

A number of reports also suggested that eukaryotes employ QQ to control colonizing or pathogenic bacteria (reviewed in reference 11). Vascular plants are thought to secrete compounds that disrupt QS, although active components have not yet been identified (11). Halogenated furanones produced by red algae appear to block QS and inhibit biofilm formation on

their surfaces, although the mechanism of inhibition is not entirely clear (6, 17, 18, 22).

Much effort is being invested in the synthesis of synthetic signal antagonists or mimics that can be applied in medicine to attenuate pathogenesis and in industry to minimize biofouling. A number of preliminary successes in this endeavor have been reported, usually where families of compounds have been designed based on known natural quorum quenchers, such as LuxR-inhibiting furanones, or peptide mimics of AIPs (10, 16, 20).

No doubt, many more potential QQ systems and mechanisms will come to light in future research, and it will be most interesting to follow these developments. In defined systems in the laboratory, many interactions may be proven to involve signal quenching. The real question will be whether or not, in the context of complex interspecies communication in nature, these systems are chance or necessity.

REFERENCES

1. **Carlier, A., R. Chevrot, Y. Dessaux, and D. Faure.** 2004. The assimilation of gamma-butyrolactone in *Agrobacterium tumefaciens* C58 interferes with the accumulation of the N-acyl-homoserine lactone signal. Mol. Plant-Microbe Interact. **17:**951–957.
2. **Carlier, A., S. Uroz, B. Smadja, R. Fray, X. Latour, Y. Dessaux, and D. Faure.** 2003. The Ti plasmid of *Agrobacterium tumefaciens* harbors an *attM*-paralogous gene, *aiiB*, also encoding N-acyl homoserine lactonase activity. Appl. Environ. Microbiol. **69:**4989–4993.
3. **Chai, Y., C. S. Tsai, H. Cho, and S. C. Winans.** 2007. Reconstitution of the biochemical activities of the AttJ repressor and the AttK, AttL, and AttM catabolic enzymes of *Agrobacterium tumefaciens*. J. Bacteriol. **189:**3674–3679.
4. **Chevrot, R., R. Rosen, E. Haudecoeur, A. Cirou, B. J. Shelp, E. Ron, and D. Faure.** 2006. GABA controls the level of quorum-sensing signal in *Agrobacterium tumefaciens*. Proc. Natl. Acad. Sci. USA **103:**7460–7464.
5. **d'Angelo-Picard, C., D. Faure, I. Penot, and Y. Dessaux.** 2005. Diversity of N-acyl homoserine lactone-producing and -degrading bacteria in soil and tobacco rhizosphere. Environ. Microbiol. **7:**1796–1808.
6. **Defoirdt, T., C. M. Miyamoto, T. K. Wood, E. A. Meighen, P. Sorgeloos, W. Verstraete, and P. Bossier.** 2007. The natural furanone (5Z)-4-bromo-5-(bromomethylene)-3-butyl-2(5H)-furanone disrupts quorum sensing-regulated gene expression in *Vibrio harveyi* by decreasing the DNA-binding activity of the transcriptional regulator protein luxR. Environ. Microbiol. **9:**2486–2495.
7. **Dong, Y. H., L. H. Wang, J. L. Xu, H. B. Zhang, X. F. Zhang, and L. H. Zhang.** 2001. Quenching quorum-sensing-dependent bacterial infection by an N-acyl homoserine lactonase. Nature **411:**813–817.
8. **Dong, Y. H., J. L. Xu, X. Z. Li, and L. H. Zhang.** 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.
9. **Fuqua, W. C., and S. C. Winans.** 1994. A LuxR-LuxI type regulatory system activates *Agrobacterium* Ti plasmid conjugal transfer in the presence of a plant tumor metabolite. J. Bacteriol. **176:**2796–2806.
10. **Geske, G. D., J. C. O'Neill, D. M. Miller, R. J. Wezeman, M. E. Mattmann, Q. Lin, and H. E. Blackwell.** 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.
11. **Hughes, D. T., and V. Sperandio.** 2008. Inter-kingdom signalling: communication between bacteria and their hosts. Nat. Rev. Microbiol. **6:**111–120.
12. **Khan, S. R., and S. K. Farrand.** 2008. The BlcC (AttM) lactonase of *Agrobacterium tumefaciens* does not quench the quorum sensing system that regulates Ti plasmid conjugative transfer. J. Bacteriol. **191:**1320–1329.
13. **Leadbetter, J. R., and E. P. Greenberg.** 2000. Metabolism of acyl-homoserine lactone quorum-sensing signals by *Variovorax paradoxus*. J. Bacteriol. **182:**6921–6926.
14. **Li, P. L., and S. K. Farrand.** 2000. The replicator of the nopaline-type Ti plasmid pTiC58 is a member of the *repABC* family and is influenced by the TraR-dependent quorum-sensing regulatory system. J. Bacteriol. **182:**179–188.
15. **Lindow, S. E., and M. T. Brandl.** 2003. Microbiology of the phyllosphere. Appl. Environ. Microbiol. **69:**1875–1883.
16. **Lyon, G. J., P. Mayville, T. W. Muir, and R. P. Novick.** 2000. Rational design of a global inhibitor of the virulence response in *Staphylococcus aureus*, based in part on localization of the site of inhibition to the receptor-histidine kinase, AgrC. Proc. Natl. Acad. Sci. USA **97:**13330–13335.
17. **Manefield, M., R. de Nys, N. Kumar, R. Read, M. Givskov, P. Steinberg, and S. Kjelleberg.** 1999. Evidence that halogenated furanones from *Delisea pulchra* inhibit acylated homoserine lactone (AHL)-mediated gene expression by displacing the AHL signal from its receptor protein. Microbiology **145:**283–291.
18. **Manefield, M., T. B. Rasmussen, M. Henzter, J. B. Andersen, P. Steinberg, S. Kjelleberg, and M. Givskov.** 2002. Halogenated furanones inhibit quorum sensing through accelerated LuxR turnover. Microbiology **148:**1119–1127.
19. **McDowell, P., Z. Affas, C. Reynolds, M. T. Holden, S. J. Wood, S. Saint, A. Cockayne, P. J. Hill, C. E. Dodd, B. W. Bycroft, W. C. Chan, and P. Williams.** 2001. Structure, activity and evolution of the group I thiolactone peptide quorum-sensing system of *Staphylococcus aureus*. Mol. Microbiol. **41:**503–512.
20. **Muh, U., B. J. Hare, B. A. Duerkop, M. Schuster, B. L. Hanzelka, R. Heim, E. R. Olson, and E. P. Greenberg.** 2006. A structurally unrelated mimic of a *Pseudomonas aeruginosa* acyl-homoserine lactone quorum-sensing signal. Proc. Natl. Acad. Sci. USA **103:**16948–16952.
21. **Pappas, K. M., and S. C. Winans.** 2003. A LuxR-type regulator from *Agrobacterium tumefaciens* elevates Ti plasmid copy number by activating transcription of plasmid replication genes. Mol. Microbiol. **48:**1059–1073.
22. **Rasmussen, T. B., M. Manefield, J. B. Andersen, L. Eberl, U. Anthoni, C. Christophersen, P. Steinberg, S. Kjelleberg, and M. Givskov.** 2000. How *Delisea pulchra* furanones affect quorum sensing and swarming motility in *Serratia liquefaciens* MG1. Microbiology **12:**3237–3244.
23. **Shelp, B. J., A. W. Bown, and M. D. McLean.** 1999. Metabolism and functions of gamma-aminobutyric acid. Trends Plant Sci. **4:**446–452.
24. **Wang, L. H., L. X. Weng, Y. H. Dong, and L. H. Zhang.** 2004. Specificity and enzyme kinetics of the quorum-quenching N-acyl homoserine lactone lactonase (AHL-lactonase). J. Biol. Chem. **279:**13645–13651.
25. **White, C. E., and S. C. Winans.** 2007. Cell-cell communication in the plant pathogen *Agrobacterium tumefaciens*. Philos. Trans. R. Soc. Lond. B Biol. Sci. **362:**1135–1148.
26. **Whitehead, N. A., A. M. Barnard, H. Slater, N. J. Simpson, and G. P. Salmond.** 2001. Quorum-sensing in gram-negative bacteria. FEMS Microbiol. Rev. **25:**365–404.
27. **Yuan, Z. C., E. Haudecoeur, D. Faure, K. Kerr, and E. W. Nester.** 2008. Comparative transcriptome analysis of *Agrobacterium tumefaciens* in response to plant signal salicylic acid, indole-3-acetic acid and γ-amino butyric acid reveals signalling cross-talk and *Agrobacterium*-plant co-evolution. Cell. Microbiol. **10:**2339–2354.
28. **Zhang, H. B., C. Wang, and L. H. Zhang.** 2004. The quormone degradation system of *Agrobacterium tumefaciens* is regulated by starvation signal and stress alarmone (p)ppGpp. Mol. Microbiol. **52:**1389–1401.
29. **Zhang, H. B., L. H. Wang, and L. H. Zhang.** 2002. Genetic control of quorum-sensing signal turnover in *Agrobacterium tumefaciens*. Proc. Natl. Acad. Sci. USA **99:**4638–4643.