GUEST COMMENTARY

Increased Chatter: Cyclic Dipeptides as Molecules of Chemical Communication in *Vibrio* spp.

Karl E. Klose*

South Texas Center for Emerging Infectious Diseases and Department of Biology,
University of Texas at San Antonio, San Antonio, Texas

Cell-to-cell communication in bacteria via chemical signal molecules has been intensively studied in the past decade, leading to an appreciation of the wide variety of mechanisms and overlapping systems bacteria use to communicate with each other and coordinate population-dependent gene expression. This phenomenon is commonly referred to as quorum sensing (QS) and has probably been best characterized in the *Vibrio* spp. Several different QS pathways have been elegantly characterized in *Vibrio fischeri*, *V. harveyi*, and *V. cholerae*; these pathways modulate bioluminescence, biofilm formation, and pathogenesis (for a review see reference 18). In the current issue, Park et al. (14a) describe a signaling molecule, cyclo-L-Phe-L-Pro (cFP) that fulfills some of the definitions of a QS molecule and may represent yet another means by which *Vibrio* spp. communicate.

QS was first described in *V. fischeri* and was shown to involve signaling through production of an acyl-homoserine lactone (AHL) molecule (14). The AHL-dependent QS systems are present in many bacterial species, and this type of signaling is believed to promote intraspecies communication, as, in general, each species makes a unique AHL that is recognized only by its cognate regulatory protein(s). The discovery of a second QS molecule in *V. harveyi*, frequently referred to as AI-2, revealed that, in this organism, parallel circuits exist and integrate two signaling pathways (AHL and AI-2 dependent) into a common response (1). AI-2 represents a family of dihydroxy-pentanedione molecules that are dependent on LuxS for synthesis, and LuxS is found in many different bacterial species (18). Thus, AI-2-dependent QS represents a mechanism for interspecies communication, and *V. harveyi* QS integrates information from both intra- and interspecies chemical signals.

Recently, third and fourth types of QS circuit have been described in *V. cholerae*. *V. cholerae* lacks an obvious AHL-dependent signaling system but has an AI-2-dependent circuit similar to that described in *V. harveyi*. In addition, *V. cholerae* has a QS signal cascade that recognizes an as yet unidentified molecule, referred to as CAI-1, that is dependent on CqsA for synthesis (11). Many, but not all, *Vibrio* spp. express CAI-1 (4). Moreover, *V. cholerae* has yet another sensory circuit that feeds information into the QS pathway through the CsrA protein (8). All three signaling cascades (AI-2, CAI-1, and CsrA dependent) converge to modulate the phosphorylation state of LuxO, and thus *V. cholerae* integrates three separate QS signals into a common sig-

nal transduction pathway, which likely allows for more-stringent control over QS-dependent behaviors (Fig. 1).

In a screen for QS molecules from *V. vulnificus* that could stimulate AHL-dependent QS reporter strains, Park et al. (14a) identified a cyclic dipeptide molecule, cFP, rather than an AHL. cFP is released into *V. vulnificus* cell-free culture fluids in a density-dependent manner, with maximum concentrations present as cells enter stationary phase. Addition of both purified and chemically synthesized cFP altered gene expression in several *Vibrio* spp., including *V. vulnificus*, *V. cholerae*, *V. parahaemolyticus*, and *V. harveyi*. The most obvious effect of cFP in the various *Vibrio* spp. is the stimulation of the expression of the major outer membrane porin OmpU. OmpU transcription is modulated by the transmembrane regulatory protein ToxR (12), which is also essential for the virulence cascade responsible for expression of the major virulence factors cholera toxin (CT) and toxin coregulated pilus (TCP) in *V. cholerae*. The authors show that cFP also stimulates CT expression in *V. cholerae*, presumably through an effect on ToxR-dependent transcription. Since OmpU is known to enhance resistance to anionic detergents, organic acids, and antimicrobial peptides (9, 10, 15) and since CT is the cause of the characteristic fluid loss that is the hallmark of cholera, the authors conclude that cFP represents a potential QS molecule that contributes to the pathogenesis of *Vibrio* spp. Whether cFP represents a “true” QS molecule awaits further experimentation to uncover the molecular mechanism behind its mode of action.

The three QS pathways previously described in *V. cholerae* that converge to modulate LuxO-phosphate production have been shown to modulate the expression of virulence factors, including CT, by altering the expression of the global regulator HapR (20). The effect of cFP appears to be distinct from these other QS pathways, because cFP stimulates ToxR-dependent transcription, while the LuxO-dependent signals affect virulence through the modulation of levels of TcpP, another virulence-regulatory factor (Fig. 1). The LuxO-dependent pathway represses CT expression at high cell density, while cFP appears to stimulate CT expression at high cell density. These two phenomena are not as distinct as they might appear, however, given the complexity of the virulence cascade. While ToxR alone activates OmpU expression and can also activate CT expression (6), ToxR and TcpP cooperatively activate the expression of ToxT, which then activates CT and TCP expression (7). Therefore a scenario could be envisioned whereby cFP exerts its effects on the established QS pathways, which leads to altered TcpP-ToxR interactions, resulting in an indirect stimulation of genes that can be activated exclusively by ToxR.

* Mailing address: Dept. of Biology, University of Texas San Antonio, 6900 N. Loop 1604 W., San Antonio, TX 78249. Phone: (210) 458-6140. Fax: (210) 458-4468. E-mail: kklose@utsa.edu.

FIG. 1. Model of quorum sensing in *Vibrio cholerae*. See text for details. Quorum-sensing pathways responsive to AI-2 and CAI-1, as well as to a third molecule that has not yet been identified, converge to modulate the level of LuxO-phosphate (LuxO-P). LuxO-P represses the expression of HapR, and HapR represses the expression of TcpP. TcpP and ToxR coordinately activate the transcription of ToxT, which in turn activates expression of the virulence factors CT and TCP. ToxR, independently of TcpP, also activates CT expression, as well as OmpU. The cyclic dipeptide cFP, which has some characteristics of a QS molecule, appears to stimulate ToxR-dependent transcription of OmpU and CT.

While this alternate explanation may seem somewhat far-fetched, cFP, along with additional cyclic dipeptides, were previously characterized for their stimulation of AHL-dependent QS in *Pseudomonas aeruginosa* and *Pseudomonas putida* by “cross talk” (3, 5). Holden et al. (5) suggested that the *Pseudomonas* cyclic dipeptides compete for the AHL binding sites on the AHL-responsive regulator LuxR. Although no AHL-dependent QS has been described in *V. cholerae*, HapR could perhaps be bound by cFP, or cFP may bind one of the established QS receptors. A more attractive possibility that fits better with the observed cFP-dependent stimulation of OmpU expression in several *Vibrio* spp. is that cFP specifically alters ToxR-dependent transcription, perhaps by interacting directly with ToxR. ToxR transcriptional activity is known to be modulated by membrane-disruptive agents such as bile (16) and high pressure (19), which is not necessarily surprising for an integral membrane transcriptional activator, but no molecules have yet been described that directly bind to ToxR.

cFP belongs to a family of cyclic dipeptides referred to as diketopiperazines (DKP), and these molecules (including cFP) have been identified as having antifungal activity (17), anti-tumor activity (2), and antibacterial activity (13). The involvement of DKP in QS adds to the plethora of potential functions of these intriguing molecules. Does the involvement of cFP in QS represent an artificial cross-reactivity with established pathways that normally respond to related compounds? Or, is cFP a broad-spectrum molecule used to modulate the activity of both eukaryotic and prokaryotic cells in a density-dependent manner,

and thus represents a novel molecule enabling interkingdom communication?

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