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From a Local Dialect to a Common Language

Quinolone-dependent cell-to-cell signaling was thought to be limited to *Pseudomonas aeruginosa*. The study by Diggle et al. in the July issue of *Chemistry & Biology* [1] now presents clear evidence that quinolone signaling molecules are produced by several bacterial species and may be used for intergenus communication.

In recent years, it has become evident that bacteria not only exist as individual cells, but often coordinate their activities and act in a concerted manner similar to that of multicellular organisms. Interactions between cells are brought about by cell-to-cell communication systems that depend on the production and detection of small diffusible signaling molecules. This phenomenon, which has been termed "quorum sensing" (QS) [2], provides bacteria with a regulatory mechanism to express certain phenotypic traits in a population density-dependent manner. To date, various signal molecules belonging to different chemical classes have been described [3]. Among gram-negative bacteria, signaling systems that rely on *N*-acyl-homoserine lactones (AHL) appear to be particularly widespread and have been identified in more than 70 species. By far the best investigated QS circuitry is the one operating in the opportunistic pathogen *Pseudomonas aeruginosa*. In this organism, two hierarchically arranged AHL-dependent QS systems (*las* and *rhl*) form a highly sophisticated regulatory network involving numerous cellular components that coordinates expression of various functions, most notably the synthesis of virulence factors (for recent reviews see [4, 5]). In 1999, Pesci et al. [6] discovered that an additional signal, 2-heptyl-3-hydroxy-4(1*H*)-quinolone, affects virulence gene expression. More detailed analyses revealed that this *Pseudomonas* quinolone signal (PQS) functions as an integral component of the QS network (reviewed in [7]). Although additional work will be required to elucidate the complex interrelationships between the two signaling pathways, it is clear that PQS production is modulated by both AHL-dependent QS systems and that exogenous addition of PQS upregulates *rhl*-controlled QS phenotypes in *P. aeruginosa* [8].

The PQS biosynthetic pathway has been studied extensively. Based on the findings that (1) *P. aeruginosa* converted radiolabeled anthranilate into radioactive PQS and (2) that an anthranilate analog (methyl anthranilate) inhibited the production of PQS, Calfee et al. [9] suggested that anthranilate is a precursor for PQS. This hypothesis was corroborated in a recent study by Bredenbruch et al. [10], who, by using isotopically labeled precursor molecules, showed that the biosynthesis of PQS and other 2-alkyl-4(1*H*)-quinolones (AHQs) proceeds through a head-to-head condensation reaction between anthranilic acid and various β-keto fatty acids.

The genes required for AHQ synthesis were initially identified in a screen for mutants defective in pyocyanin synthesis [11]. The *pqsABCDE* operon contains five genes that encode for a putative coenzyme A ligase (*pqsA*), two β-keto-acyl-acyl carrier protein synthases (*pqsB*, *pqsC*), and a FabH1 homologous transacetylase (*pqsD*). The *pqsE* gene appears to encode a response effector protein which itself is not involved in the biosynthesis of PQS. Although the exact functions of the enzymes remain to be elucidated, it is clear that the *pqsABCD* gene products direct the synthesis of 2-heptyl-4-quinolone (HHQ), the immediate precursor of PQS. HHQ is thought to be an extracellular messenger that is released from and taken up by *P. aeruginosa* cells ([12], Figure 1). Once taken up, HHQ is converted into PQS by the action of the putative FAD-dependent monoxygenase PqsH. Expression of the *pqsH* gene, which is not physically linked to the *pqsABCD* operon, is partially controlled by the *las* system, connecting AHL-dependent QS with PQS signaling [11].

Quinolone signaling was thought to be unique to *P. aeruginosa*, since PQS molecules could not be detected in the culture supernatants of several other *Pseudomonas* species [13]. However, in the July issue of *Chemistry & Biology*, Diggle et al. [1] presented convincing evidence that quinolone-dependent signaling is more widespread than so far anticipated. To assay for AHQ production, the authors developed a simple and rapid method for screening bacterial culture supernatants. To this end, a *P. aeruginosa* bioreporter was constructed, which cannot synthesize AHQs due to inactivation of the *pqsA* gene, but which responds to exogenously supplied AHQs with the emission of light. This biosensor strain can be incorporated within agar and used as an overlay following TLC of the solvent-extracted culture supernatants.

Using this approach as a fast initial screen and LC-MS/MS for confirmation of the molecules' identity, it was shown that several *Burkholderia pseudomallei* strains, *Burkholderia cenocepacia*, *Burkholderia thailandensis*, and *Pseudomonas putida* produce HHQ, but not PQS. Examination of the currently completed bacterial genomes revealed that *B. pseudomallei* and *B. thailandensis* each contain a complete, putative *pqsABCDE* operon (designated *hhqABCDE*), while in *P. putida* the genes for HHQ synthesis are scattered throughout the chromosome. In agreement with the chemical analysis, none of the strains contained an apparent *pqsH* homolog.

For *P. aeruginosa*, it has been suggested that HHQ functions as a messenger molecule which has to be converted to PQS before it can act as a signaling molecule [12]. This hypothesis is based mainly on the observation that addition of PQS to the culture medium of the wild-type strain induced certain functions prematurely while exogenous provision of HHQ had no effect [8]. This observation is in strong contrast to the case of *B. pseudomallei*, in which mutation of the *pqsA* homolog resulted not only in the loss of HHQ production, but also in altered colony morphology and increased elastase production [1]. These results suggest that, at least in *B. pseudomallei*, HHQ serves as a signaling molecule per se, and it raises the question of whether HHQ can function

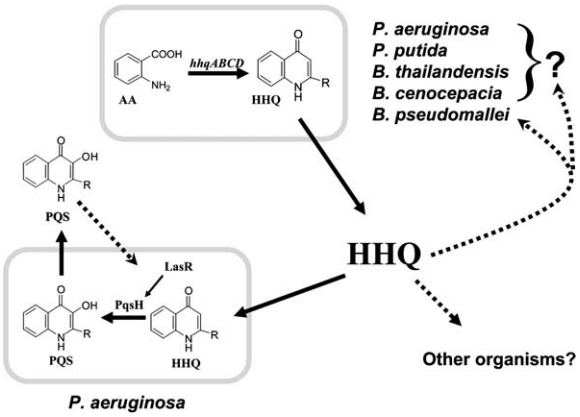

Figure 1. Quinolone-Dependent Signaling in Bacteria

Several bacterial species are capable of synthesizing HHQ, which is used as a signal molecule by *B. pseudomallei* and possibly other bacteria. HHQ is taken up by cells of *P. aeruginosa* and is then converted to PQS by the action of PqsH, expression of which is partly QS regulated. Solid arrows indicate release and uptake of quinolones, and dashed arrows depict the known and putative regulatory activities of signaling molecules. PQS not only serves as a signal molecule for *P. aeruginosa* but is also an iron chelator. R: C7 alkyl chain.

similarly in *P. aeruginosa.* It is tempting to speculate that PQS is a highly specialized dialect of *P. aeruginosa* that is not "spoken" by many other bacteria, whereas HHQ is a more common language used for intergenus communication. In this context, it is also worth mentioning that PQS is a selective iron chelator [14], suggesting that conversion of HHQ to PQS may serve a function in *P. aeruginosa* that is not related to signaling, but which may be important for the fitness of the organism in the environment.

While *B. pseudomallei* and *B. cenocepacia* are important human pathogens, *B. thailandensis* and *P. putida* are nonpathogenic soil saprophytes. *B. pseudomallei* is the causative agent of melioidosis, an often fatal infection of animals and humans, and *B. cenocepacia* is an important opportunistic pathogen in both cystic fibrosis patients and immunocompromised individuals [15]. Is it possible that crosstalk between such diverse organisms could occur? Intriguingly, all of these bacteria are commonly isolated from plant roots, and it is therefore conceivable that they may coexist in the rhizosphere of plants. Whether these bacteria indeed form mixed aggregates on root surfaces and whether under these circumstances AHQs are used for intergenus signaling remains to be investigated. It is also noteworthy that all AHQ-producing species also produce AHL signaling molecules, which were previously shown to be used for communication between bacteria in the rhizosphere [16]. Hence, these organisms may utilize more than just one chemical language to converse with each other. In *P. aeruginosa*, the AHL- and AHQ-dependent signaling pathways are closely interlinked [4, 5], and it will be interesting to see whether such an overlap also exists in other bacteria. Moreover, as AHQ-dependent signaling is required for the regulation of virulence in *P. aeruginosa*, another future challenge will be to elucidate the importance of this signaling pathway for virulence gene regulation in *B. pseudomallei* and *B. cenocepacia*.

Given that AHQs are synthesized from key cellular metabolites, it appears likely that this class of signaling molecules is widely used by bacteria. With the AHQ biosensor developed in this study, an ideal tool is now available to explore the full extent and diversity of AHQ-mediated signaling between bacteria.

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Selected Reading

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