Point of VIEW

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Bacterial Signal Destruction

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ABSTRACT Many bacteria use a molecule known as autoinducer-2 for interspecies communication, a form of quorum sensing. Enteric bacteria secrete this molecule and later import and degrade it. A new study explores the molecular mechanism behind this curious signal-destroying process.

In many bacteria, behaviors such as bioluminescence, biofilm formation, antibiotic production, conjugation, and virulence are performed only when the cell density is high, a process known as quorum sensing (*1, 2*). First identified in bioluminescent members of the genus *Vibrio*, quorum sensing is now appreciated to be a widespread phenomenon in bacteria. The molecular basis of quorum sensing is the secretion and detection of signaling molecules called autoinducers. The autoinducer concentration is used by the bacteria as a measure of their population density (Figure 1). Each bacterial cell produces a low level of autoinducer, and upon reaching a threshold concentration, the autoinducer is bound by cognate receptors that control gene expression (*1, 2*). The autoinducer-2 (AI-2) molecule regulates a variety of functions in different bacteria, including the transport and degradation of the AI-2 molecule itself in enteric bacteria (*3–5*). On page 128 of this issue, Xavier *et al.* (*6*) explore the molecular basis of AI-2 degradation.

The autoinducer AI-2 was originally identified as one of two quorum-sensing autoinducers in *Vibrio harveyi* (*1*). AI-2 deviates from the general quorum-sensing paradigm in three ways: (i) AI-2 is a collection of interchangeable molecules derived from 4,5-dihydroxy-2,3-pentanedione (DPD), rather than a single compound; (ii) DPD is a product of the activated methyl cycle, and therefore AI-2 synthesis is intimately tied to metabolism; and (iii) AI-2 and its synthase, LuxS, are found in numerous, evolutionarily diverse bacteria, an indication that AI-2 mediates interspecies communication.

AI-2 Is a Collection of Molecules Derived from DPD. LuxS catalyzes the biosynthesis of DPD, the precursor to the various molecules collectively known as AI-2 (Figure 2, top; *1, 7, 8*). DPD readily undergoes spontaneous cyclization and hydration to form several different interchangeable products (*7–9*). The X-ray crystal structures of the AI-2 receptors of *V. harveyi* and *Salmonella typhimurium* bound to their respective ligands revealed that these two organisms detect different DPD-derived products. *V. harveyi* detects the product of borate addition to *S*-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran (*S*-THMF–borate) (*7*), whereas *S. typhimurium* detects *R*-THMF (Figure 2, top; *8*). Thus, a single precursor gives rise to an assortment of molecules out of which an AI-2 receptor can select a single compound. Whether other bacteria recognize other derivatives of DPD remains to be discovered.

DPD Is a Product of the Activated Methyl Cycle. The activated methyl cycle involves the transfer of the methyl group of methionine, *via S*-adenosylmethionine, to various substrates (Figure 2, bottom; *10–14*). This process is critical for the biosynthesis of certain amino acids, nucleic acids, lipids, and proteins in all organisms (*10*). LuxS catalyzes the formation of DPD and homocysteine from *S*-ribosylhomocysteine (*9, 13, 15*). Thus, DPD, in addition to being a precursor to AI-2 molecules, is a product of cellular metabolism (*4, 9, 14, 16*).

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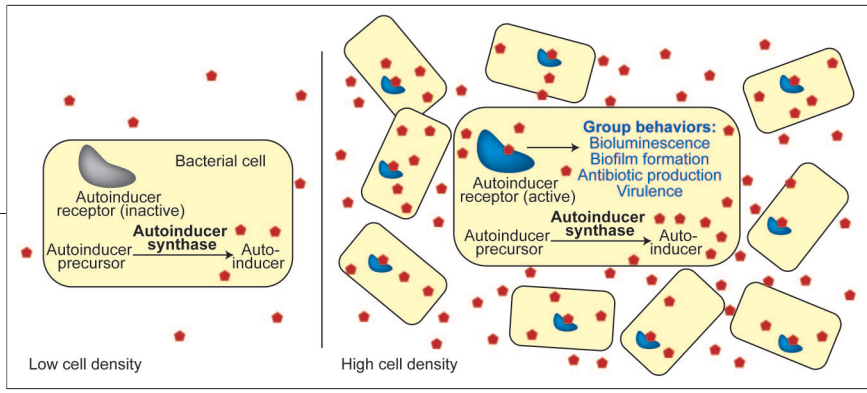

Figure 1. Quorum-sensing paradigm. Each cell contains an autoinducer synthase for production of autoinducer (red) and an autoinducer receptor for signal transduction. At low cell density (left panel), the autoinducer concentration is low, and the autoinducer receptor does not bind autoinducer. At high cell density (right panel), when a threshold concentration of autoinducer is exceeded, autoinducer binds its receptor and triggers the expression of density-dependent functions, including the examples listed in blue. The autoinducer receptor can be either cytoplasmic or membrane-associated.

AI-2 Functions as a Quorum-Sensing Autoinducer in Some Bacteria. Because of the widespread distribution of LuxS in bacteria, AI-2 has been proposed to function in interspecies communication (*17, 18*). Several reports confirm that LuxS, DPD, or both control virulence, biofilm formation, and other behaviors in a number of bacterial species (for review, see refs 3 and 4). DPD mediates mixed-species biofilm development among oral pathogens, confirming that DPD functions as an interspecies autoinducer (*19*). In pure culture experiments, behaviors that would provide obvious benefits in mixed-species environments, such as antibiotic production and biofilm formation, have been shown to be activated by DPD (*3, 4*). In addition, DPD controls the expression of several virulence factors in *Pseudomonas aeruginosa*, which itself does not produce AI-2 (*20*). These and other findings confirm that interspecies communication by AI-2 is widespread.

Not all bacteria that contain LuxS homologues control gene expression in response to DPD, and this suggests a separate metabolic role for LuxS. For example, growth of a *luxS* mutant of *Staphylococcus aureus* is impaired, suggestive of a metabolic problem (*21*), and in some bacteria, the effects of *luxS* inactivation cannot be rescued by addition of DPD (*4, 21*). It is possible that AI-2 signaling evolved from its original role as a metabolite to control gene expression in some bacteria, whereas in others LuxS retains only its primary metabolic role and is not involved in signaling (*4*). Nevertheless, AI-2 production apparently is used by some bacteria to indicate the presence of others.

Transport and Degradation of DPD by Enteric Bacteria. Curiously, in the enteric bacteria *Escherichia coli* and *S. typhimurium*, DPD induces the expression of genes that encode an ATP binding cassette transport apparatus called LuxS regulated (Lsr), which imports DPD into the cell (Figure 2, middle) (*5, 22*). Lsr kinase (LsrK) phosphorylates DPD upon entry into the cell (*23*). Lsr repressor (LsrR) regulates *lsr* transcription, and genetic evidence suggests that phosphorylated DPD (P-DPD) functions as the antirepressor of LsrR (*5, 23*). Also encoded in the *lsr* operon are a putative aldolase, LsrF, and a small protein of unknown function, LsrG. These two proteins were proposed to function in the degradation of P-DPD (*23*). A recent report showed that transport and degradation of DPD by the Lsr system can effectively block AI-2 signaling of other bacteria (*24*). However, the chemical reactions involved and the physiological function of the Lsr system remain unknown.

Xavier *et al.* (*6*) provide the critical molecular details associated with the regulation, phosphorylation, and degradation of DPD. The LsrK-catalyzed reaction of DPD with ^{32}P-labeled ATP and the subsequent LsrG-catalyzed reaction were followed by thin layer chromatography. The structures of the phosphate-containing compounds in this pathway were determined by mass spectrometry, ^{1}H and ^{31}P NMR, and 2D NMR. The authors show that DPD is phosphorylated by LsrK at C5, thereby trapping DPD in the ring-open conformation (Figure 2, middle). P-DPD is then cleaved by LsrG to form 2-phosphoglycolic acid (PG) and a molecule of unknown structure. This work also demonstrates that P-DPD directly binds LsrR, providing a molecular explanation for the LsrK-dependent derepression of *lsr* transcription by LsrR.

What, then, is the "intended" function of the Lsr transport and degradation system? One possibility is that the Lsr system is a mechanism of "quorum quenching" and is therefore functionally analogous to the *N*-acylhomoserine lactone (AHL) lactonases and acylases employed by a number of bacteria that interfere with the quorum-sensing systems of other bacteria (*24–28*). Consistent with this possibility, *E. coli* can use the Lsr system to interfere with AI-2 signaling in mixed-species cultures (*24*). However, a quorum-quenching role would predict that the phylogenetic distribution of the Lsr system would be independent from LuxS, yet the Lsr system is found nearly exclusively in bacteria containing LuxS (*13*). The Lsr system could instead be a mechanism that enteric bacteria use to quench their own quorum-sensing signaling. This role would be analogous to the AHL acylases of *P. aeruginosa* that specifically degrade an endogenously produced autoinducer, possibly as a mechanism of terminating group behaviors such as biofilm formation (*29, 30*). In this scenario, DPD in enteric bacteria would control an unidentified set of genes in these organisms. An alternative function for the Lsr system could be a DPD-scavenging mechanism. DPD would be used as an energy source when nutrients become limiting, analogous to the "acetate switch" in which acetate excreted during rapid growth is later internalized and metabolized (*31*). A role for the Lsr system in DPD scavenging is supported by the finding of Xavier *et al.* (*6*) that DPD is cleaved to form PG, which can be metabolized by enteric bacteria (*6, 32*). However, such a role would predict that DPD could be used as an energy source, yet this

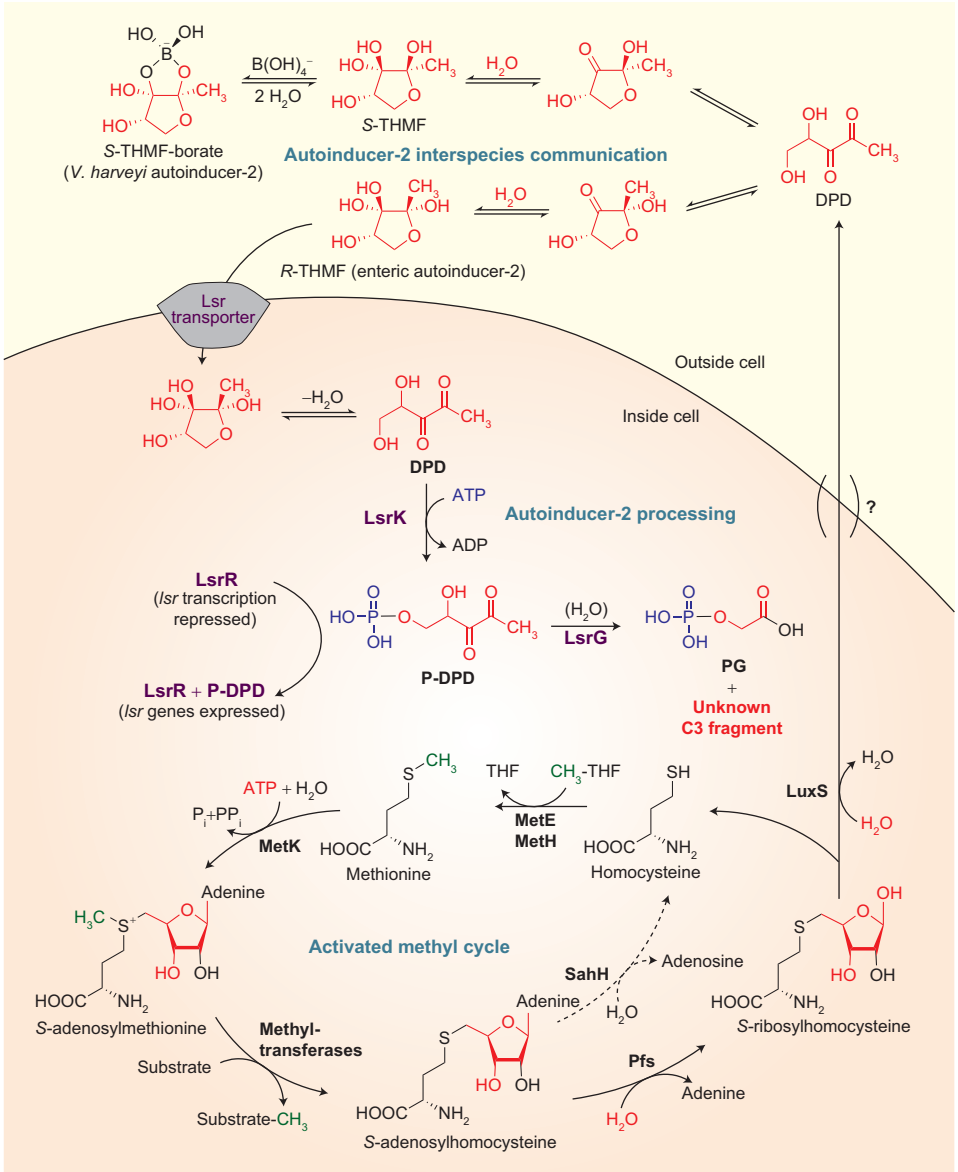

Figure 2. DPD synthesis, transport, and degradation in enteric bacteria. DPD synthesis is a product of the activated methyl cycle, in which methyl donation (green) by *S*-adenosylmethionine to various substrates results in the formation of the toxic intermediate *S*-adenosylhomocysteine (SAH). In eukaryotes and some bacteria, SAH is hydrolyzed in one step to produce adenosine and homocysteine (dashed arrows). Organisms that contain a LuxS homologue break down SAH in two steps, the first catalyzed by Pfs to form *S*-ribosylhomocysteine and adenine and the second catalyzed by LuxS to form homocysteine and the AI-2 precursor DPD (red). Methionine is regenerated from homocysteine in an N5-methyltetrahydrofolate (CH_3-THF)-dependent reaction. DPD exits the cell by an unknown mechanism. DPD spontaneously undergoes cyclization and hydration to form either *R*- or *S*-THMF. Borate addition to *S*-THMF results in the formation of *S*-THMF–borate, the interspecies AI-2 signal perceived by *V. harveyi*. *R*-THMF is imported by the Lsr transporter of enteric bacteria. Once transported into the cell, DPD is phosphorylated (blue) by LsrK to form C5-phospho-DPD (P-DPD). P-DPD is cleaved by LsrG, possibly by hydrolysis, to form PG and an unknown three-carbon compound.

is not the case in *S. typhimurium* and has not been investigated in other bacteria (*5*). Identification of the other products of DPD degradation could provide further clues about the relationship between the Lsr degradation system and metabolism. Regardless of the original role of the Lsr system, its ability to destroy DPD could be exploited as a mechanism to combat the numerous pathogens that employ AI-2 signaling for virulence.

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