Bacterial Quorum Sensing: Signals, Circuits, and Implications for Biofilms and Disease

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Key Words

Abstract

Communication between bacteria, belonging to the same species or to different species, is mediated through different chemical signals that are synthesized and secreted by bacteria. These signals can either be cell-density related (autoinducers) or be produced by bacteria at different stages of growth, and they allow bacteria to monitor their environment and alter gene expression to derive a competitive advantage. The properties of these signals and the response elicited by them are important in ensuring bacterial survival and propagation in natural environments (e.g., human oral cavity) where hundreds of bacterial species coexist. First, the interaction between a signal and its receptor is very specific, which underlies intraspecies communication and quorum sensing. Second, when multiple signals are synthesized by the same bacterium, the signaling circuits utilized by the different signals are coordinately regulated with distinct overall circuit architecture so as to maximize the overall response. Third, the recognition of a universal communication signal synthesized by different bacterial species (interspecies communication), as well that of signals produced by eukaryotic cells (interkingdom communication), is also integral to the formation of multispecies biofilm communities that are important in infection and disease. The focus of this review is on the principles underlying signal-mediated bacterial communication, with specific emphasis on the potential for using them in two applications—development of synthetic biology modules and circuits, and the control of biofilm formation and infection.

Contents

1. INTRODUCTION

Communication between bacteria, belonging to the same species or to different species, is mediated through different chemical signals that are synthesized and secreted by the various bacteria. These signals can either be related to cell density or population (quorum-sensing signals) (1) or simply signals produced by bacteria at different stages of growth (e.g., indole, which is produced by *Escherichia coli* during the stationary phase of growth) (2). An important difference between the two types of signals is that quorum-sensing signals, collectively known as autoinducers, are utilized by bacteria for cell-cell communication in a concentration-dependent manner (i.e., high cell density) (1), whereas other bacterial signals are not constrained by cell density requirements.

Quorum-sensing systems were originally discovered in the marine bacterium *Vibrio fischeri* as being involved in the control of light production (3), and have been identified in a wide range of bacterial genera, including *Pseduomonas*, *Escherichia*, and *Streptococcus*. Although bacteria can detect a signal at any concentration, autoinducer-mediated communication often occurs in a concentration-dependent manner. This population effect is utilized by bacteria to regulate phenotypes in a manner that enables them to adapt and survive continually changing environments by coupling individual cell responses to population-wide alterations. For example, the Gram-positive *Streptococcus pneumoniae* upregulates the expression of genes involved in the production of antimicrobial peptides only when the concentration of signaling molecules in the culture medium increases above a critical threshold concentration (4, 5). On the other hand, other bacterial signals can also be utilized in cell-cell communication without being involved in population-wide regulation of traits and phenotypes. The origin of these signals differs—some signals (e.g., indole) may be produced by bacteria as part of their normal metabolism, whereas others (e.g., hydroxyindole) may be produced by bacteria modifying these signals and utilizing them for regulating different phenotypes.

The focus of this review is primarily on quorum sensing–mediated cell-cell communication in bacteria. We first describe the different quorum sensing signals and the structure of quorum-sensing circuits used in various Gram-negative and -positive bacterial species for communication. We then discuss the specificity of signal-mediated cell-cell communication between bacteria belonging to the same species, different species, as well as different kingdoms (i.e., between

prokaryotes and eukaryotes). We also discuss cell-cell signaling mediated by non-quorum-sensing signals, such as indole, and, finally, elaborate on applications of signal-mediated communication in synthetic biology and in controlling the formation of bacterial biofilms.

2. QUORUM-SENSING SIGNALS AND CIRCUITS

Quorum sensing is almost always utilized to control various bacterial phenotypes with extremely high specificity and exquisite control. The level of specificity and control is intriguing given the complexities associated with microbial communities: Bacteria are almost always present in multispecies communities [e.g., oral cavity biofilms have been reported to contain nearly 400 bacterial species (6)], each bacterial species can produce multiple signals [e.g., *Vibrio harveyi* has three autoinducer signaling systems (7)], and the response circuits utilized by these signals are also interconnected (1). Therefore, it is not surprising that quorum-sensing signals have high specificity for their cognate receptors (i.e., they are primarily recognized only by the bacterial species that produces them). Similarly, quorum-sensing circuits have also evolved to prioritize signal information and respond only to specific signals to preserve the fidelity of the induced response. Quorum-controlled processes such as the production of light by *V. fischeri*, the expression of virulence determinants by *P. aeruginosa*, or the downregulation of EsaR of *Pantoea stewartii* occur only when high-cell-density conditions are attained (8). This section focuses on the different signals produced and signaling circuits utilized by different bacteria for functioning in complex multispecies environments.

2.1. Quorum-Sensing Systems

The *V. fischeri* quorum-sensing circuit is prototypical of most quorum-sensing systems present in Gram-negative bacteria (9). This basic quorum-sensing circuit consists of two regulatory proteins—LuxI and LuxR—that function in the synthesis and recognition of the autoinducer, respectively, to control production of light. LuxI is the enzyme that synthesizes an autoinducer *N*-(3-oxohexanoyl)-homoserinelactone (HSL) that can diffuse in and out of the cell. At low cell densities, a small amount of LuxI protein is present, which leads to low levels of the autoinducer signal and light production through expression of luciferase genes (10). As the cell density increases, the concentration of the autoinducer increases both inside and outside the cell. When the signal concentration reaches a threshold level (11), the autoinducer binds to the LuxR protein and activates it by exposing a DNA binding domain. The activated LuxR binds to the promoter region of the *luxCDABE* operon to upregulate, among others, transcription of the luciferase genes and the production of light. This quorum-sensing circuit is shown in **Figure 1**.

Because *luxI* is also one of the genes that are upregulated by the activated LuxR signal, quorum sensing also leads to further production of LuxI and thereby a rapid increase in the production of light. However, signal production (i.e., *luxI* expression) does not continually increase; the positive feedback amplification of light production is balanced by negative feedback regulation of the *luxR* gene by the activated LuxR protein. This, in turn, leads to a decrease in LuxR levels, production of LuxI, and the expression of luciferase genes.

Quorum sensing in almost all Gram-negative bacteria is mediated through a regulatory circuit that is analogous to the above-described *V. fischeri* LuxI/LuxR system. For example, in the opportunistic pathogen *P. aeruginosa*, the *lasI* gene encodes for an autoinducer synthase (LasI) that leads to synthesis of homoserine lactone autoinducer, whereas the *lasR* gene encodes for the response regulator (LasR) (12–14). Similar to *V. fischeri*, LasR binds to the autoinducer signals and regulates the expression of target genes. A second quorum-sensing system in *P. aeruginosa*
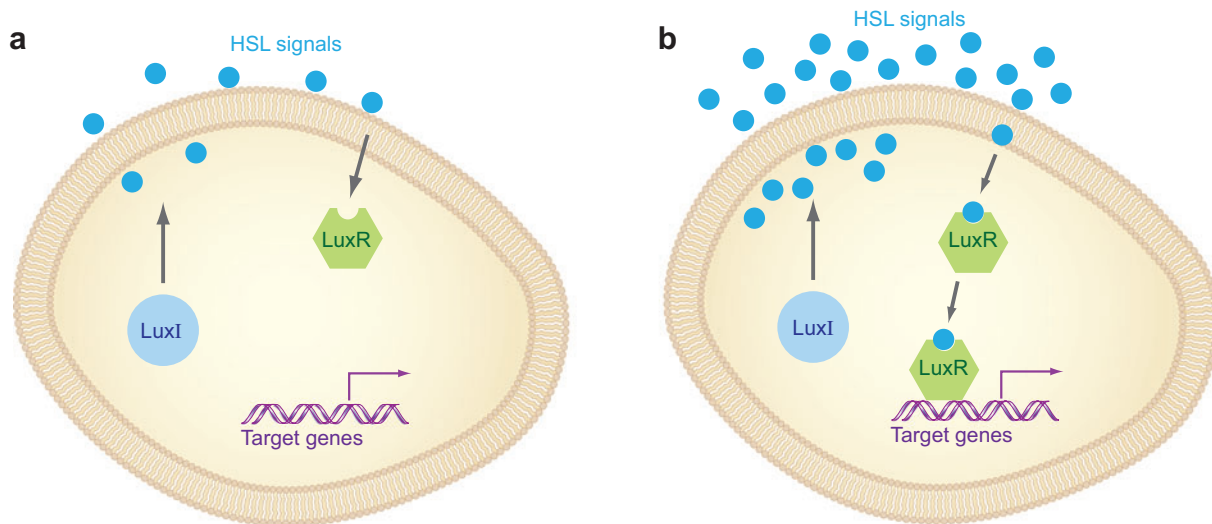
Figure 1

Quorum sensing with diffusible signals. Acyl-homoserine lactone (HSL) signals (*blue circles*) are produced by the LuxI enzyme homologues that bind to LuxR homologues to activate expression of target genes. (*a*) At low cell densities, concentration of the signal is low both inside and outside the cell, with minimal activation of LuxR. (*b*) At high cell densities, acyl-HSL activates LuxR through binding and leads to expression of downstream target genes.

(RhlI/RhlR) also functions through a similar circuit to regulate expression of target genes (14). The target genes regulated in *P. aeruginosa* by the LasI/LasR and RhlI/RhlR systems include those encoding virulence determinants such as elastase and proteases that play important roles in infection. Similar quorum-sensing systems homologous to *luxI* and *luxR* are found in other bacterial species, including the lung pathogen *Burkholderia cepacia* (15, 16), the enteric pathogen *Yersinia enterocolitica* (17), and the plant pathogen *Agrobacterium tumefaciens* (18). These quorum-sensing systems encode a diverse range of functions, including siderophore production (19), cell division (20), polysaccharide synthesis (21), and motility (15). Although quorum-sensing systems have been identified in several Gram-negative bacterial species, the range of functions controlled by quorum sensing in these bacteria is not fully understood.

Quorum-sensing systems in Gram-positive bacteria differ from the canonical acyl-HSL-mediated quorum sensing in Gram-negative bacteria in the structure of the autoinducer molecules as well as in the mechanism of signal recognition and sensing (**Figure 2**). The autoinducer molecule used in Gram-positive bacteria is a peptide (autoinducing peptide, AIP) (1, 22) in contrast to the acyl-HSL signal used in Gram-negative bacteria. Also, AIP signals do not freely diffuse in and out of cells; instead, they are synthesized as precursor peptides, modified, and exported from cells using protein transport machinery (23). Sensing and recognition of the AIP occurs not by direct binding to a cognate receptor but through a two-component signal transduction system, in which the AIP binds to a membrane-bound histidine kinase sensor and the binding information is relayed to the cell through phosphorylation of response regulator proteins that ultimately bind to the promoter of target genes to regulate gene expression (22, 24). The *S. pneumoniae* circuit is a classic example of quorum-sensing regulation in Gram-positive bacteria, with the *comAB* genes involved in exporting peptide signals, *comC* producing the AIP, *comD* functioning as the AIP receptor, and *comE* functioning as the intracellular response regulator. Similar systems have been identified in other Gram-positive species such as *Bacillus* and *Staphylococcus*. Interestingly, the
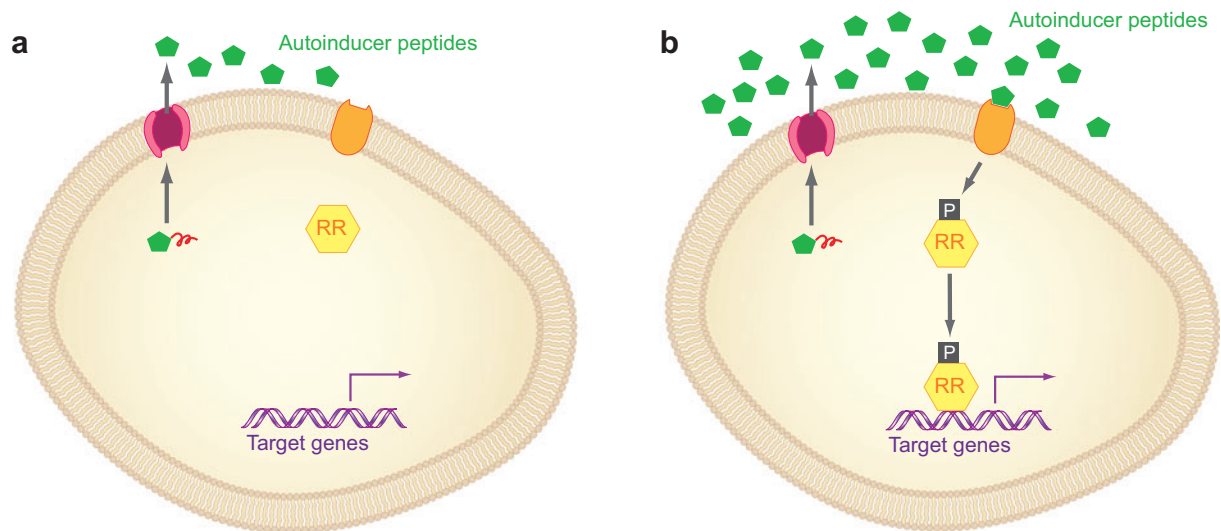
Figure 2

Quorum sensing with autoinducer peptides (AIPs) (*green pentagrams*). AIPs are produced as precursor peptides and exported out of the cell. (*a*) At low cell densities, concentration of the AIP signal is low outside the cell and there is no activation of the response regulator (RR). (*b*) At high cell densities, binding of the AIP to a histidine kinase receptor leads to phosphorylation of the RR and expression of downstream target genes.

mechanisms utilized in the Gram-positive two-component signal transduction system are similar to the autoinducer-2 quorum-sensing system found (in addition to the canonical LuxI-LuxR system) in the Gram-negative *V. harveyi* (1, 25).

2.2. Autoinducer Signals and Cognate Receptors

Different Gram-negative bacteria produce different acyl-HSL signals that activate the respective quorum-sensing circuits. These autoinducer signals (**Figure 3**) are typically composed of a HSL core with different acyl groups attached to it. Acyl-HSL autoinducers are synthesized by the LuxI autoinducer synthase (or its homologues) using *S*-adenosylmethionine (SAM) and acyl-acyl carrier proteins as substrates in a reaction that involves formation of an amide linkage between SAM and acyl groups, and subsequent lactonization to the autoinducer signal (26). The HSL core is conserved between the different autoinducer signals, and diversity in signals is simply achieved through differences in the acyl side chain groups attached to the HSL core (27, 28).

LuxR-like proteins function as receptors for the different autoinducer signals and together with the signal, regulate the expression of target genes (29). The two functions of LuxR-like proteins—binding of the autoinducer signal and binding to the promoter regions of target genes—are typically achieved through two separate domains. Autoinducers primarily bind to the amino-terminal end of LuxR [acyl-binding domains (30, 31)], whereas the carboxy-terminal end is involved in DNA binding to target gene promoter regions. The segregation of autoinducer-binding and DNA-binding domains has significant implications for the functioning of LuxR as the cognate receptor for quorum-sensing signals. It has been shown that the autoinducer-binding domain masks the DNA-binding activity of the C-terminal domain when the autoinducer is not present, and this interference is removed upon autoinducer binding (30). This mode of regulation ensures that the expression of target genes is not altered without activation of LuxR (i.e., not before the
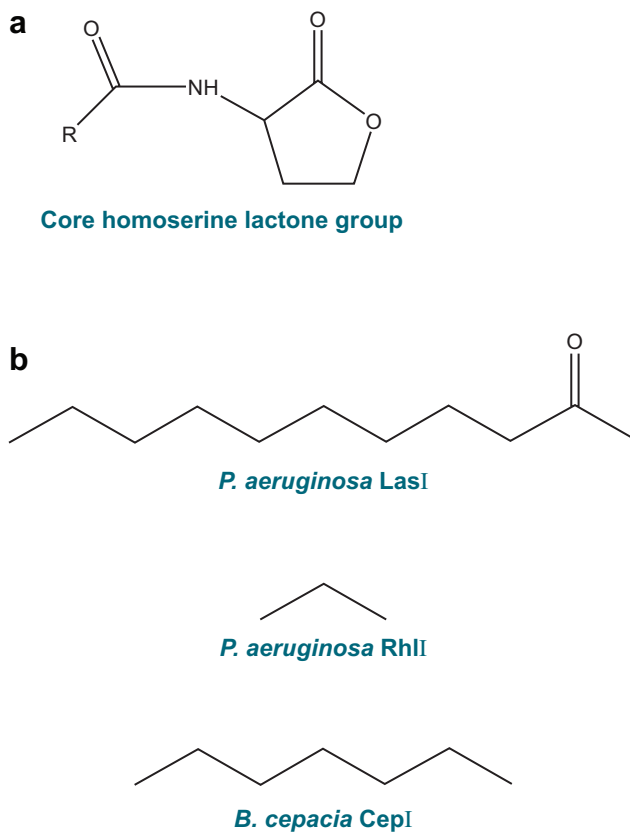
a

Core homoserine lactone group

b

P. aeruginosa LasI

P. aeruginosa RhlI

B. cepacia CepI

Figure 3

Structures of acyl homoserine lactone signals. (*a*) Core homoserine lactone group that is common to this class of signals. (*b*) Different side chain moities (R groups) that are linked to the core molecule in different bacteria. Note that cross-talk occurs between *P. aeruginosa* and *B. cepacia* despite the differences between the LasI and CepI signals produced by these bacteria, respectively.

autoinducer signal reaches a threshold value). Similarly, autoinducer binding to LuxR has also been shown to alter stability of the LuxR protein. Prior studies with TraR, the LuxR homologue in *A. tumefaciens*, have shown that TraR is not properly folded and is susceptible to proteolytic degradation in the absence of autoinducers. However, binding of autoinducers to TraR alters its conformation, thereby increasing its resistance to protelytic degradation. Interestingly, TraR stabilization by folding in the presence of autoinducers occurs only in nascent polypeptides and not with preexisting TraR (32).

2.3. Specificity of Autoinducer Signals

A key feature of autoinducer-mediated signaling in quorum sensing is the high degree of specificity for the signal to its cognate receptor (LuxR-like protein). Because bacteria are invariably found in multispecies communities (e.g., the oral cavity, gastrointestinal tract), it is extremely important for bacteria to be able to discriminate between autoinducer signals produced by their own species and those produced by other bacterial species present in the environment. This is especially important in the case of pathogenic bacteria, where the expression of different virulence genes needs to be

coordinated for infection, and signal interference through other signals in the environment can strongly impact the extent of infection.

Specificity of autoinducer-mediated signaling can be achieved either through specificity in binding interactions between each autoinducer signal and its LuxR-like protein receptor or through regulation of activated LuxR binding to the promoter of target genes. Several studies have shown that fidelity of acyl-HSL-mediated signaling is primarily achieved through specificity in the activation of LuxR-like proteins by the autoinducers rather than through DNA binding of activated LuxR-like proteins. This is because the DNA-binding domain of LuxR-like proteins, such as the 20–base pair sequence in the promoter region of target genes [i.e., the lux box (33)], is highly conserved. Therefore, discrimination between signals is not likely to be carried out at the level of DNA binding by activated cognate receptors but rather at the stage of activation of LuxR-like proteins (i.e., binding of autoinducer signals to LuxR-like proteins).

Because the binding between autoinducer signals and its cognate receptor is an important determinant of signal specificity (34), the composition and structure of the signals are also important in maintaining specificity of signaling. Structural analysis of cognate receptor-acyl-HSL pairs indicates that the acyl side chain of autoinducer signals is a key determinant of the specificity observed in autoinducer signaling. Differences in the length, structure, and substitutions in the acyl side chain groups (35) can impact the binding between autoinducers and LuxR-like proteins, and thereby quorum sensing–regulated signaling. The specificity of the signal synthase for selected side chain moieties ensures that only specific types of signals are produced in different species. For example, the LuxI homologues EsaI in the plant pathogen *Pantoea stewartii* (36) and LasI in *P. aeruginosa* produce 3-oxo-C_6-HSL and 3-oxo-C_{12}-HSL, respectively, and this specificity has been linked to specific amino acid residues in the side chain binding pocket of the two enzymes. A threonine at residue 140 of EsaI appears to be necessary for 3-oxo-C_6-HSL production in *P. stewartii* as a threonine-to-alanine substitution results in the production of other acyl-HSL molecules with different side chain lengths and substitutions (37). Interestingly, the production of the original 3-oxo-C_6-HSL was not changed, indicating that this amino acid ensures signal specificity by allowing the addition of a specific side chain moiety. A similar shift in specificity, although less pronounced, was also observed with LasI from *P. aeruginosa* containing a specific amino acid substitution (37). These studies clearly demonstrate that the range of signals being produced in each species is tightly regulated.

A similar regulatory scheme is also utilized in Gram-positive bacteria for autoinducer specificity. Most AIP signals (**Figure 4**) have a core peptide whose sequence is not conserved and can be modified differently by different Gram-positive bacteria (38). Specificity in the recognition of AIP signals is primarily controlled through recognition of the AIP signal (24, 39). However in the case of AIP signaling, this specificity is achieved through binding of the signal and the sensor kinase on the cell surface (as opposed to specificity between the signal and the cognate response regulator in Gram-negative bacteria), as the AIP signal is not internalized in Gram-positive bacteria.

2.4. Specificity with Multiple Signals and Cross-Talk

Although there is high specificity between a specific autoinducer signal and its cognate receptor, several bacterial species produce more than one signal and possess multiple quorum-sensing circuits. For example, *V. harveyi* produces three autoinducer signals—an acyl-HSL molecule HAI-1 (3-hydroxy-butyl HSL), a furanosyl borate diester (auotinducer-2 or AI-2), and a second acyl-HSL known as CAI-1, which was recently identified as (*S*)-3-hydroxytridecan-4-one—that use different cognate receptors for regulating quorum-controlled responses such as luminescence and biofilm formation (7). Multiple quorum-sensing signals are produced in several other
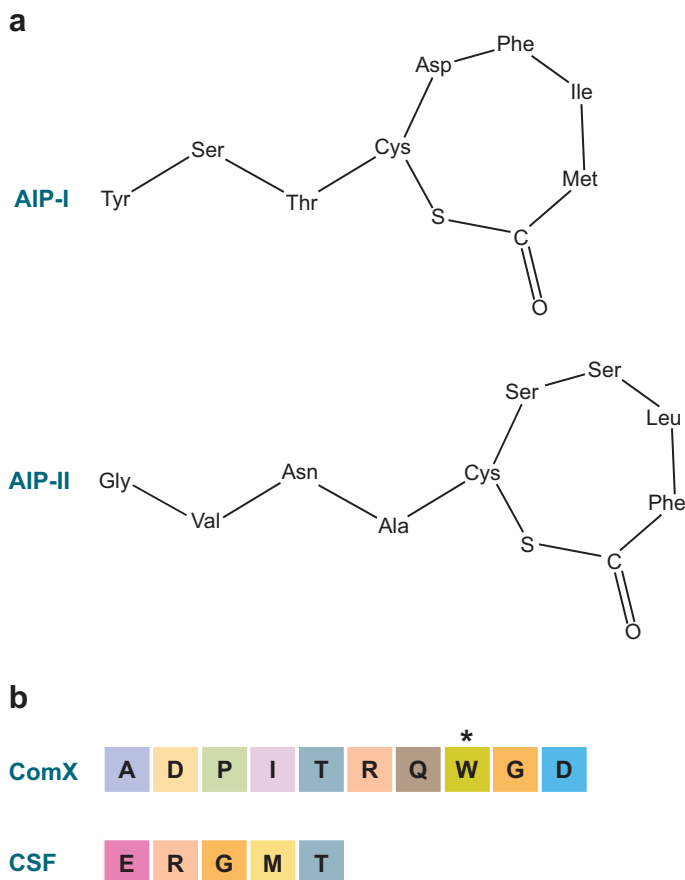
Figure 4

Structures of autoinducer peptide (AIP) signals. (*a*) AIP from *S. aureus*. Note that the core peptide sequence is not conserved. (*b*) ComX and CSF peptides used for quorum sensing in *B. subtilis*. * indicates modified amino acid residue.

bacterial species, including *P. aeruginosa* (40) and *S. aureus* (41). These signals need not be similar; in *P. aeruginosa*, two acyl-HSL autoinducers (3-oxo-C_{12} and C_4 HSL) are produced in addition to quinolone-based signals (42).

Despite the structural constraints and regulatory mechanisms involved in ensuring the specificity of quorum sensing, some degree of nonspecific signaling or cross-talk has been observed in Gram-negative and -positive bacteria and occurs through both recognition and processing of autoinducer signals. Nonspecific signal-mediated interactions include recognition of a signal produced by a different bacterium or interference with processing of autoinducer signals through competition (43). An example of the former is the interaction between *P. aeruginosa* and *Burkoholderia cepacia* (44) that are found in lungs of cystic fibrosis patients. Researchers have shown that quorum-sensing cross-talk occurs between the two bacteria but is unidirectional. The C_4 and 3-oxo-C_{12} homoserine lactones produced by *P. aeruginosa* are recognized by *B. cepacia* at low concentrations to activate its *cep* quorum-sensing system; however, *P. aeruginosa* is not capable of utilizing *B. cepacia* acyl-HSL to activate its *las* or *rhe* quorum-sensing systems. This acyl-HSL-cross-talk has significant implications for the development of mixed-species biofilms and the pathogenesis

of cystic fibrosis as *P. aeruginosa* colonization precedes *B. cepacia* in cystic fibrosis patients, and suggests that nonspecific quorum sensing could be one mechanism adopted by *B. cepacia* to develop a multispecies community with *P. aeruginosa* and propagate infections. Other examples of recognition of a signal produced by another bacterium include the increase in biofilm formation by *P. aeruginosa* in response to indole produced by *E. coli* (*P. aeruginosa* does not produce indole) and reduction in biofilm formation by *E. coli* in response to acyl-HSL produced by *P. aeruginosa* (*E. coli* cannot produce acyl-HSLs) (2).

The lack of specificity in signaling has also been observed in the Gram-positive *S. aureus*, where AIP produced by one strain of *S. aureus* interferes with other *S. aureus* in addition to upregulating virulence genes in its own species (39, 45). Interestingly, this interference did not significantly impact cell growth, but it did disrupt the activation of virulence genes. These observations suggest that QS-based interference can be used by pathogens for creating a niche for a particular strain during infections (i.e., only a strain that produces a specific AIP can compete and survive in a particular environment). Similar observations have also been made by Firth et al. (46), who demonstrated that a *Staphylococcus* lipoprotein product is recognized as a pheromone by *Enterococcus faecalis*.

However, not all interspecies signal-mediated communication needs to be nonspecific, as many bacterial species also produce signals that are specifically used for signaling to other bacterial species (i.e., interspecies communication). The acyl-HSL and AIP signals are primarily intended and used by bacteria for communication with their own species, and not between two different species. However, because bacteria often exist in multispecies communities, communication and cooperation (e.g., metabolic cooperation) between different species (47, 48) is required for establishment and sustenance of the microbial community. To date, only one signal has been known to function as an interspecies cell-cell communication signal. Originally identified in *Vibrio harveyi* as a regulator of luminescence (49), the *luxS* gene involved in the synthesis of autoinducer-2 (AI-2) (**Figure 5**) has been identified in more than 55 species of Gram-negative and -positive bacteria (50). Consistent with its classification as a universal cell-cell communication signal, AI-2 has been found to have a role in interspecies communication in several environments, including the oral cavity and the gastrointestinal tract.

The role of AI-2 in oral cavity biofilms and disease has been well characterized. The early oral cavity colonizer *Streptococcus gordonii* (51), as well as the pathogen associated with the etiology of dental caries, *Streptococcus mutans* (52), have the *luxS* gene needed for AI-2 synthesis. McNab et al. (53) have shown the importance of AI-2 in the colonization of a *S. gordonii* biofilm by the pathogen *Poryphyromonas gingivalis*. Although a *P. gingivalis luxS* mutant demonstrated reduced expression of virulence genes, it was still able to form a mixed species biofilm with wild-type *S. gordonii* and unable to do so with a *S. gordonii luxS* mutant. The organization of multispecies biofilm communities has also been shown to be regulated through AI-2 signaling. For example, Rickard et al. (54) and McNab et al. (53) have shown that interaction between different oral bacteria and biofilm formation is mediated through AI-2 signaling.

Although AI-2 has been primarily described as an interspecies signal, it also appears to be involved in intraspecies signaling and regulation of phenotypes. Our prior work, as well as that from other laboratories (55, 56), has shown that AI-2 acts as a signal even when a single species is present. The importance of AI-2 in intraspecies signaling and the control of bacterial motility have been demonstrated in several species, including *E. coli* K-12, *Helicobacter pylori*, *Aggregatibacter actinomycetemcomitans*, and *Campylobacter jejuni*. AI-2 stimulates biofilm formation and changes its architecture by stimulating flagellar motility via the quorum-sensing regulator MqsR, which acts through the two-component motility regulatory system QseBC (55). This result is consistent with the recent finding that AI-2 regulates biofilm formation in *A. actinomycetemcomitans*, most likely
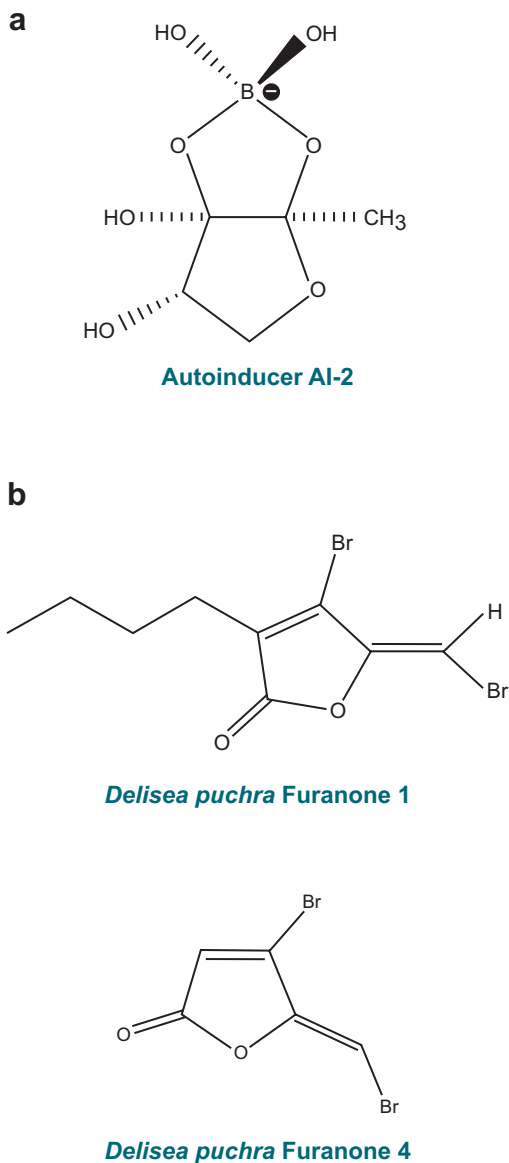
Autoinducer AI-2

Delisea puchra **Furanone 1**

Delisea puchra **Furanone 4**

Figure 5

Structures of the interspecies quorum-sensing signal autoinducer-2 and its antagonists. (*a*) Autoinducer-2 (AI-2) from *V. harveyi*. (*b*) Furanones produced by the red algae *Delisea puchra* that inhibit AI-2 activity.

through its QseBC system (56). AI-2 also controls motility in *H. pylori* and *C. jejuni* by controlling genes upstream of the motility and flagellar regulator (57) and the transcription of flagellin genes (58), respectively. Although these reports clearly indicate that AI-2 is a key intraspecies signal in bacterial motility, recent results from our laboratory (59) also show that AI-2 regulates the expression of virulence genes that are involved in *E. coli* O157:H7 infections. Together, these observations suggest an emerging role for AI-2 as an intraspecies signaling molecule in addition to its well-characterized function in interspecies signaling.

2.5. Quorum-Sensing Circuit Architecture

Although the existence of multiple quorum-sensing signals and response circuits is well established, their organization and the functions that they coordinately regulate in different bacteria are not fully understood. For example, the circuits utilized for signaling with the acyl-HSL signals are well characterized in *P. aeruginosa*, but the quorum-sensing circuits utilized by quinolone signals are not fully understood. An important paradigm that is emerging is that the different quorum-sensing circuits need not be completely independent; instead, they can be coupled to one another in a manner that enables the bacteria to respond maximally when presented with different signals and environments.

Quorum-sensing circuits can be organized with different architectures in different bacteria. The pathways responding to the three autoinducer signals in *V. harveyi* are arranged in parallel, as each pathway can be independently activated by different signals, yet, they all converge to provide information to the same pathway and phenotypes (e.g., luminescence) (**Figure 6a**). In *V. harveyi*, the LuxO protein is the common intermediate through which all three quorum-sensing systems exert their effects (7). From a network perspective, this arrangement can be beneficial as it provides a way for cells to synchronize their responses to the different signals (60). Moreover, a parallel quorum-sensing circuit architecture and the requirement for multiple signals to be present can also serve to tightly regulate the quorum-sensing response as nonspecific activation of cellular responses is minimized (i.e., the probability that multiple circuits are erroneously activated simultaneously is very small).

Unlike *V. harveyi*, the Las and Rhl quorum-sensing circuits are arranged in series in *P. aeruginosa*, where each quorum-sensing system regulates the expression of different sets of genes in a
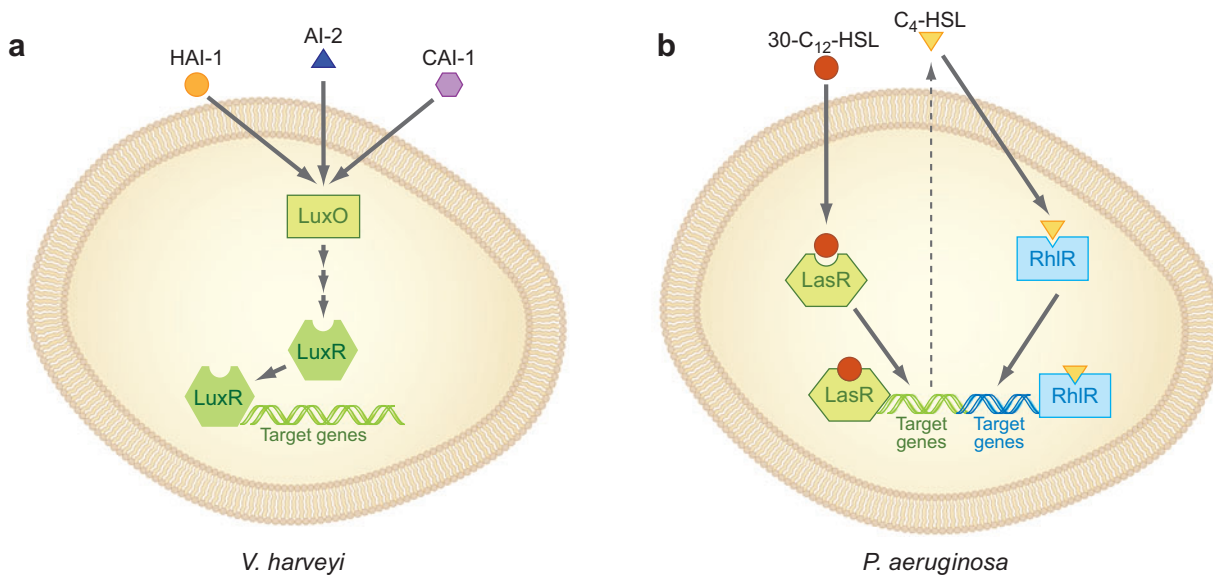

Figure 6

Architecture of quorum-sensing circuits. (*a*) Multiple quorum-sensing systems are arranged in parallel in *V. harveyi*, where all three signals (AI-1, AI-2, CAI-1) converge on LuxO, which activates LuxR through a series of steps and results in the regulation of target genes. (*b*) The two main quorum-sensing systems in *P. aeruginosa* are arranged in series. One signal (C_{12}-HSL) activates a set of target genes, including the synthase for producing the second signal (C_4-HSL). This signal, in turn, activates a different set of target genes. Some overlap also exists between the two sets of target genes. Simplified versions of both circuits, with only pertinent molecules, are shown.

temporally defined manner (**Figure 6b**). Under high-cell-density conditions, the Las system is initially induced and results in the synthesis of the autoinducer molecule 3-O-C_{12}-HSL (61). This signal binds to and activates its cognate receptor LasR, which in turn upregulates the expression of several genes, including *lasI* (positive feedback). Two of the target genes for activated LasR are the autoinducer synthase (RhlI) and cognate regulator of the Rhl quorum-sensing system (RhlR) (13). Thus, activation of LasR leads to synthesis of a different autoinducer signal and activation of the second quorum-sensing system. Because the Las system is activated prior to the activation of the Rhl system, these *P. aeruginosa* quorum-sensing systems are thought to be arranged in series. *P. aeruginosa* also responds to the signal 2-heptyl-3-hydroxy-4-quinolone (PQS), which has been postulated to act as a link between the Las and Rhl quorum-sensing systems (62). PQS production has also been shown to depend on the relative levels of the other two *P. aeruginosa* signals (3-O-C_{12}-HSL and C_4-HSL), suggesting that the balance between different quorum-sensing systems is important (63).

The distinct temporal arrangement of quorum-sensing systems ensures that only certain genes are expressed at a given time during the course of *P. aeruginosa* infections (64). However, it should also be noted that not all genes and processes are regulated by a single acyl-HSL; several studies have demonstrated that some *P. aeruginosa* genes respond to either 3-O-C_{12} or C_4-HSL, as well as to both acyl-HSL molecules (65, 66), which suggests additional levels of network complexity in the *P. aeruginosa* quorum-sensing circuits. However, not all quorum-sensing systems act in unison, as quorum-sensing circuits have also been shown to counter each other (i.e., the different quorum-sensing systems regulate the expression of different phenotypes that are distinct and may counteract one another). An example of this is seen in *B. subtilis* where one AIP (ComX) regulates the onset of competence (67), while a second AIP (CSF) (68) interferes with ComX signaling and promotes sporulation (69).

3. INTERKINGDOM SIGNALING

The close association of nonpathogenic bacteria with eukaryotic cells has led to the notion that cell-cell communication mechanisms may also exist between bacteria and host cells. This is primarily based on the fact that large numbers of bacteria are present in close proximity to host cells as part of the native human microflora. For example, $\sim 10^{14}$ commensal bacteria are present in the human gastrointestinal tract (70, 71), and more than 500 bacteria species are thought to be present in the human oral cavity (6, 72). Therefore, it is not surprising that some form of communication underlies their coexistence in complex environments (73, 74). Moreover, the intimate attachment of bacteria to eukaryotic cell surfaces during the development of certain infections [e.g., *P. aeruginosa* colonization in the lung (75), *A. tumefaciens* colonization and crown gall tumor production in plants (76)] also suggests that communication between pathogenic bacteria and host cells is important.

In the context of interactions between pathogens and eukaryotic cells, it has been shown that pathogens can respond to host signals (77–79) and host cells can also recognize signals produced by pathogenic bacteria (77, 80). The enteric pathogen *E. coli* O157:H7 is an excellent example of a pathogen utilizing intra- and interkingdom signaling for infections (81). Because the infective dose of *E. coli* O157:H7 is extremely low ($\sim$100 CFU mL^{-1}) (82), it has been speculated that this pathogen relies almost exclusively on utilizing other bacterial or host signals present in the gastrointestinal tract. Indeed, our recent work has shown that *E. coli* O157:H7 chemotaxis, motility, colonization, and gene expression are all altered upon exposure to the eukaryotic hormones norepinephrine and epinephrine (81). Although the exact pathways activated by norepinephrine and epinephrine are not known, there is evidence to suggest that the bacterial autoinducer-3 (AI-3)

signaling pathway is involved as these hormones can replace AI-3 to activate the expression of genes involved in virulence (83–85). These studies suggest that hormones in the gastrointestinal tract may cross-talk with bacterial quorum-sensing pathways. The implications of such cross-talk in enteric infections are significant as the pathogen can utilize different signals at different stages of infection depending on which signal is dominant in the environment.

Interactions between bacteria and plant-derived signals have also been reported. The *Agrobacterium* spp. have been shown to migrate toward signals released from plant wounds [e.g., phenolic compunds (86)] to initiate the cell-cell contact required for infection. The bacteria bind to tumor-derived molecules such as opines (87), which results in expression of the LuxR-like protein TraR. When TraR is activated, it upregulates replication of a tumor-inducing plasmid and its transfer into plant cells (88). Similarly, bacteria have also been shown to utilize the major plant hormone indole 3-acetic acid as a source of carbon, nitrogen, and energy (89). Hence, prokaryotes utilize eukaryote signals to recognize specific hosts and even to initiate disease states.

Interkingdom signaling between prokaryotes and eukaryotes leads to competition and interference of cell signals. One of the best-studied examples of interkingdom signaling interference is in the blocking of quorum sensing by the quorum-sensing inhibitor brominated furanone from the seaweed *Delisea pulchra*; this algae produces compounds such as (5*Z*)-4-bromo-5-(bromomethylene)-3-butyl-2(5*H*)-furanone (**Figure 5b**) that inhibit quorum sensing and thereby inhibit social behavior like biofilm formation (90, 91). We recently showed the brominated furanone interferes with all three quorum-sensing systems in *V. harveyi* by affecting the last protein (master regulator protein LuxR) after convergence of the three quorum-sensing pathways and rendering it unable to bind to the promoter sequences of quorum sensing–activated genes (92). Another example of interkingdom interference is the inactivation of acyl-HSL bacterial signaling via lactonases present in sera (93).

4. INDOLE SIGNALING

Apart from quorum sensing–based signals, other types of cell-cell signaling molecules are also important in the context of bacterial phenotypes and infections. One such signal is indole, a relatively new bacterial signal that has been postulated to be the archetypal hormone of eukaryotes (2). Extracellular indole is found at high concentrations (over 600 μM) when *E. coli* is grown in rich medium (94, 95), and was identified initially as a stationary-phase signal that controls six groups of genes (96, 97). Recently, indole signaling has been shown to link plasmid multimerization and cell division (98), and shown to be a nontoxic interspecies signal that decreases biofilm formation in *E. coli* (2). In a manner analogous to acyl-HSL signals that bind SdiA (99, 100) and control biofilm formation in *E. coli* (2), indole controls biofilms by inducing the acyl-HSL sensor of *E. coli*, SdiA, which influences cell motility and acid resistance (2), even though *E. coli* does not produce acyl-HSL signals (100). Hence, the protein *E. coli* uses to monitor signaling of acyl-HSL-producing bacteria is necessary to monitor its own indole signaling. Beyond biofilms, indole has also been shown to control multidrug exporters in nonpathogenic *E. coli* K-12 (101); to regulate the pathogenicity island of enteropathogenic *E. coli* (102); and to repress *gadX* of *E. coli* K-12 (2), which activates virulence in EHEC (103). Recent work from our laboratory has also shown that indole signaling is a crucial determinant of *E. coli* O157:H7 infections, as it repels the pathogen, decreases motility, attenuates adherence to epithelial cells, and downregulates the expression of genes related to virulence and infection (81).

Hydroxy derivatives of indole have also been found to be bacterial signals. Based on the competition for various cell signals and given that indole controls biofilms (2) and is present

at high concentrations (95), we hypothesized that hydroxylated indoles may play a role in biofilm formation because many bacterial oxygenases, such as those found in *Burkholderia cepacia* G4 (104), readily convert indole to oxidized compounds, such as 2-hydroxyindole, 3-hydroxyindole, 4-hydroxyindole, isatin (indole-2,3,-dione), indigo, isoindigo, and indirubin (104). We found that hydroxyindoles and isatin are interspecies biofilm signals that affect EHEC, *E. coli* K-12, and *P. aeruginosa* PAO1 by controlling biofilm-related genes, including AI-2 transport, indole synthesis, flagellar synthesis, and cysteine regulation (105). Specifically, it appears isatin mimics AI-2 for stimulating biofilm formation (97) because both induce the same flagellar genes. Isatin also repressed AI-2 importers (*lsr* operon) and repressed indole synthesis. 7-Hydroxyindole was found to decrease biofilms primarily through changes in sulfur and purine metabolism. Because indole signaling is mediated by SdiA (which also recognizes acyl-HSLs), the implications of these results are that in *E. coli*, AI-2-, indole-, and acyl-HSL–based signaling are all interconnected, so these signals seem to be working in parallel.

5. APPLICATIONS OF QUORUM SENSING

5.1. Synthetic Biology

An emerging application of quorum-sensing circuits is in synthetic biology. Synthetic biology involves the engineering of artificial networks of proteins and/or metabolites to impart new functions to cells (106–108). Although conceptually similar to genetic engineering, it differs from the former by "the use of modularity, abstraction, and standardization to allow generalized principles and designs to be applied to different scenarios" (109). For example, the entire protein machinery involved in the expression of a specific protein—from the promoter region of a gene and the proteins involved in generating mRNA, to the translation site and the ribosomal machinery needed to generate polypeptides—together can be thought of as comprising a basic module that accepts an input and produces an output in the form of a specific protein. These modules function similar to logic gates and can be used to perform logic operations such as NOT (110) (i.e., function as a biological inverter where presence of a signal leads to expression of a repressor protein for a specific target gene). Synthetic biology approaches have been recently used for achieving several functions, including gene-metabolite oscillations (111), programming population control (112), genetic clock and toggle switch (113, 114, 115), coupling natural and engineered gene networks (116), and in spatiotemporal control of gene expression (117).

Cell-cell communication, especially quorum sensing, is relevant to synthetic biology as the development of synthetic circuits and networks draws heavily upon basic tenets of cell-cell communication, such as the specificity of interaction between a signal and its cognate receptor. The interaction between cell-cell communication and synthetic biology is clearly two-way, as synthetic biology approaches can also help understand complex phenomena and observations in cell-cell communication. An example of this is the synthetic multicellular bacterial system with positive and negative feedback regulation of signaling described by Basu et al. (117). In this network, sender cells were engineered to transmit different acyl-HSL signals to nearby pulse-generating receiver bacteria with a feed-forward module that responds to the signal by expressing green fluorescent protein. Using this system, the authors demonstrated that cells grown on solid media exhibit distinct spatiotemporal characteristics, such as response of receiver cells mainly to nearby, but not distant, sender cells. Such synthetic pulse-generators have potential applications in developmental biology, as the system behaves similar to that governing the formation of dorsal appendages in *Drosophila melanogaster* (118).

5.2. Control of Biofilm Formation

Most bacteria in nature are not present as free-floating isolated cells; instead, they are often found associated with surfaces (119, 120). These microbial communities, or biofilms, are composed of bacteria that are enmeshed within an exopolysaccharide matrix. The bacteria are not randomly distributed in the polysaccharide matrix, but are present as highly organized structures that enable transport of nutrients and waste in and out of the biofilm. Moreover, biofilms in nature are rarely comprised of a single bacterial species, and several species often are present in the biofilm community. For example, oral cavity biofilms (or dental plaque) are thought to be made of hundreds of species that coordinately form the biofilm in a spatiotemporally regulated manner (6, 72, 121). Biofilms are an extremely important clinical problem, as biofilm bacteria exhibit very high resistance to antimicrobial agents and host immune defense mechanisms (120). Therefore, understanding the mechanisms underlying biofilm formation could lead to approaches for controlling biofilm formation.

The exquisite organization of biofilm communities to support the survival and lifestyle of different bacterial species is the hallmark of biofilms. In the context of dental plaque, bacterial species that are either aerobic or tolerate oxygen stress are the initial colonizers of tooth surfaces, whereas anaerobic bacteria colonize only after sufficiently thick biofilms have developed and anaerobic pockets are established (72). Cell-cell communication and quorum sensing is one of the mechanisms that has been proposed to govern the development and sustenance of biofilm communities and organization (122, 123). Therefore, interfering with cell-cell communication has been proposed as an attractive alternative for disrupting biofilm formation.

Given the progress made in identifying proteins related to biofilm formation (2, 55, 81, 105, 124–127), especially in *E. coli*, it is now possible to conceive of methods to control biofilm formation through manipulation of specific proteins. As an example, we discuss strategies for the control of *E. coli* biofilms. Based on our discovery that SdiA mediates biofilm inhibition by the signal indole (2), recent work in our laboratory focuses on altering SdiA to create a protein switch that can be manipulated through addition of a signal of our choosing (e.g., indole, acyl-HSL). The choice of these signals is driven by the fact that indole is nontoxic and is readily imported by *E. coli* (via Mtr), whereas acyl-HSL signals are not produced by *E. coli* but work with SdiA. As the protein regulator that responds to the chosen signals and then controls transcription, SdiA (240 aa) is a good choice because it is a DNA-binding protein that is known to interact with several acyl-HSLs (99, 100, 128), including 3-oxo-*N*-octanoyl-L-homoserine lactone (OOHL). In addition, we have shown *N*-butyryl-*DL*-homoserine lactone (10 μM, BHL) decreases biofilm formation of *E. coli* (25%) but does not change biofilm formation of the SdiA mutant (2); hence, BHL may be used as signal with SdiA.

By using a random protein-engineering approach (129), we have evolved SdiA to respond to control biofilm formation upon addition of acyl-HSLs and upon addition of indole (T.K. Wood, unpublished). To date, screening 4577 mutants in the presence of indole and two acyl-HSLs has led to the discovery of five interesting mutants. Biofilm formation by variant 1E11 was fivefold lower than that in the wild-type cells, and biofilm formation of variant 2D10 was twofold greater in the presence of OHL, while variant 6B12 showed lower biofilm in the presence of OHL. These results show clearly that mutation of SdiA can further decrease biofilm formation in *E. coli*, and SdiA variants can be constructed that recognize the interspecies signal acyl-HSL and influence biofilm formation for *E. coli*.

In addition, the authors have also constructed the first synthetic signaling circuit that controls biofilm formation (2). Recognizing indole as an interspecies signal that affects both *E. coli* and pseudomonads, and that hydroxylation of indole would alter its affect on *E. coli* biofilms, we

engineered *P. fluorescens* to overexpress toluene *o*-monooxygenase (TOM) from *Burkholderia cepacia* G4 (129) to control extracellular indole concentrations. Because indole inhibits biofilm formation, expression of TOM and the conversion of indole to insoluble indigoids (104) removed the biofilm inhibitor, which led to a 12-fold enhancement in *E. coli* cells in dual-species biofilms when the *P. fluorescens* cells removed indole via TOM versus the dual culture in which TOM was not expressed by the pseudomonad. In addition, there was a 22-fold reduction in extracellular indole in the dual culture when TOM was expressed (2). An engineered circuit based on acyl-HSL has also been recently constructed, but it was not used to control biofilm formation, instead, it was used to monitor cell signaling as visualized by expression of fluorophores (130).

Lu & Collins (109) have also recently described a synthetic biology approach for dispersal of *E. coli* biofilms using an enzymatic bacteriophage. Two approaches that have been used to disrupt biofilms: (*a*) killing bacteria using specific bacteriophages [e.g., T4 (131, 132)] and (*b*) degrading the exopolysaccharide matrix using specific enzymes (133). The modular approach described by Lu & Collins (109) combines both these approaches for more effective biofilm eradication. The *E. coli* bacteriophage T7 was engineered to express the enzyme dispersin B (DspB) to degrade the exopolysaccharide matrix. When introduced into *E. coli* biofilms, the bacteriophage replicated within biofilm cells and expressed DspB in high concentrations. Lysis of the biofilm bacteria also resulted in high local concentrations of phage and DspB leading to effective dispersal of both *E. coli* biofilm components.

6. SUMMARY AND FUTURE DIRECTIONS

It is becoming increasingly evident that cell-cell communication and quorum sensing are integral to understanding bacterial behavior relating to several clinically important issues, such as biofilm formation and infection. Several seminal studies on quorum sensing and signal-mediated communication have delineated the molecules involved as well as the network or circuit through
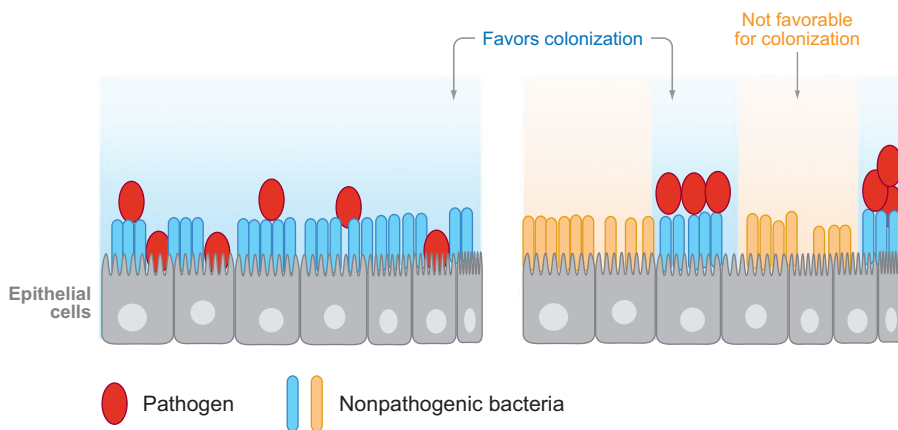

Figure 7

Signaling interaction–based colonization model. In vivo environments, such as the gastrointestinal tract, have an abundance of different signals, not all of which exert similar effects on colonization. This leads to a signal-mediated colonization model where pathogen (e.g., EHEC) colonization will occur if it encounters a favorable signal (e.g., the hormone norepinephrine). However, because the gastrointestinal tract nonpathogenic flora is not uniform, it is also likely that the pathogen encounters a signal that inhibits colonization (e.g., indole). If the pathogen encounters both signals simultaneously, the extent of colonization will depend on which signal exerts a more dominant effect on colonization.

which signal synthesis and recognition are regulated. Whereas these studies have primarily focused on individual signals, not much is known about interactions between different signals, and the next challenge involves utilizing this information in an integrative manner to describe the effect of multiple signals with divergent effects on quorum sensing circuits and communication. This is especially important considering that several bacterial species coexist in the human body (e.g., gastrointestinal tract) and that pathogens are likely to encounter different signals simultaneously; therefore, the extent of colonization and infection is likely to depend on the signal(s) encountered and the effects elicited by the different signals (promotes or attenuates virulence). This hypothesized signal-mediated colonization model is described in **Figure 7**. Moreover, recent studies showing that signal-mediated communication is not restricted to occurring between the same bacterial species, but extends to exchange of signals between different bacterial species, as well as between bacteria and host cells, also opens up new paradigms in signal-mediated cell-cell communication. The information generated from these studies has potential applications in a wide variety of scenarios, including the development of synthetic gene networks and modules, antimicrobial therapeutics, and biotechnology applications.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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