

Interkingdom signaling: Deciphering the language of acyl homoserine lactones

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Received 17 August 2004; received in revised form 7 February 2005; accepted 4 March 2005

First published online 28 April 2005

Abstract

Bacteria use small secreted chemicals or peptides as autoinducers to coordinately regulate gene expression within a population in a process called quorum sensing. Quorum sensing controls several important functions in different bacterial species, including the production of virulence factors and biofilm formation in *Pseudomonas aeruginosa* and bioluminescence in *Vibrio fischeri*. Many gram-negative bacterial species use acyl homoserine lactones as autoinducers that function as ligands for transcriptional regulatory proteins. Several recent reports indicate that bacterial acyl homoserine lactones can also affect gene expression in host cells. Direct signaling also appears to function in the opposite direction as some eukaryotic cell types produce mimics that interact with quorum sensing systems in bacteria. Here, we will describe the evidence to support the existence of bi-directional interkingdom signaling via acyl homoserine lactones and eukaryotic mimics and discuss the potential molecular mechanisms that mediate these responses. The functional consequences of interkingdom signaling will be discussed in relation to both pathogenic and non-pathogenic bacterial–host interactions.

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Keywords: Quorum sensing; Acyl homoserine lactone; Autoinducer; Interkingdom signaling; Eukaryotic AHL receptor; AHL mimics

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1. Introduction

“First learn the meaning of what you say, and then speak.”

Epictetus (55AD–135AD) Roman (Greek-born) slave & Stoic philosopher

To communicate effectively, it is important to first understand the language being spoken. This statement refers not only to communication between humans but is equally applicable to individual cells in a biological organism or population. The primary components of the language of cell–cell communication in metazoan organisms are hormones, which effectively pass information from cell to cell, often over significant distances. The discovery of the phenomenon of quorum sensing (QS), a cell density-dependent signaling mechanism used by many species of bacteria, provided the clearest evidence that this mode of communication is not restricted to eukaryotes. QS in many gram-negative bacteria utilizes small lipid-based molecules termed acyl homoserine lactones (AHLs) to communicate within and sometimes across species. A functional QS system is crucial for several important functions in different bacterial species, including biofilm formation and virulence in the pathogenic bacterium *Pseudomonas aeruginosa*, and bioluminescence in *Vibrio fischeri*. AHLs exhibit structural similarities to many eukaryotic hormones and a growing number of reports have documented apparent biological effects of AHLs on eukaryotic cells. The reverse communication conduit also appears to be open as mammalian hormones can also interact with components of the QS machinery. Bearing in mind the extended period over which bacteria and eukaryotes have co-existed, it is perhaps not too surprising that the ability to decipher cross-kingdom signals has evolved. Understanding this bi-directional language will undoubtedly have profound effects on our understanding of our relationship to our prokaryotic cousins and our abilities to continue to co-exist with, or respond to, benign or pathogenic bacteria, respectively. In this review, we will describe the evidence supporting the existence of communication

between prokaryotes and eukaryotes, which, in deference to its relationship to QS, we have termed global sensing.

2. Quorum sensing: communication within and between kingdoms

Quorum sensing was first characterized in *V. fischeri* and *Vibrio harveyi*, two bioluminescent marine symbionts of some fish and squids (reviewed in [1]). Subsequently, QS systems have been described in a large and growing number of bacterial species that control multiple cellular functions [2]. Three different major classes of QS systems have been described that utilize different classes of secreted autoinducer molecules (see Fig. 1). Many gram-negative bacteria utilize AHLs that either diffuse or are transported across cell membranes and function as ligands for a family of inducible transcription factors [3]. Gram-positive bacteria utilize small secreted peptides that activate two-component signaling pathways (Fig. 1) [2]. The third class of QS system appears to be shared by gram-positive and gram-negative bacteria and utilizes a signaling molecule initially referred to as autoinducer-2 (AI-2, reviewed in [4]). AI-2 activity in *V. harveyi* was originally attributed to a furanosyl borate diester ((2*S*,4*S*)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran-borate or *S*-THMF-borate, see Fig. 1) [5] but subsequent studies suggested that AI-2 activities may be attributed to a group of structurally related compounds whose synthesis is dependent on the LuxS enzyme. This concept has been validated by the recent determination of the structure of a distinct form of the AI-2 signal from *Salmonella typhimurium* ((2*R*,4*S*)-2-methyl-2,3,3,4-tetrahydrofuran or *R*-THMF, see Fig. 1) [6]. We will refer to this system as the LuxS/AI-2 QS system. The LuxS/AI-2 system functions by activating two-component signal transduction pathways and has been proposed as a universal system for bacterial communication within and between species [4]. QS systems, as their name implies are generally considered to control cell functions in response to alterations in cell

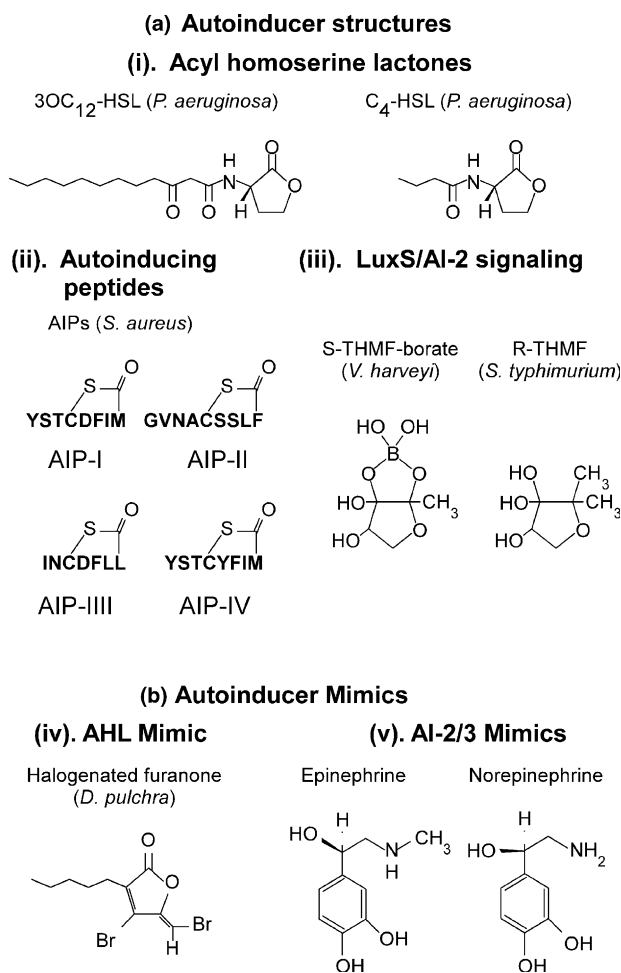


Fig. 1. Structural representations of autoinducers and selected autoinducer mimics. (a) Representative example of three classes of autoinducers: (i) acyl homoserine lactones (AHLs) used by many gram-negative bacteria. Two AHLs produced by *P. aeruginosa* are shown. (ii) Autoinducer peptides (AIPs) used by some gram-positive bacteria. Four peptides produced by *S. aureus* are shown. A thioester bridge links the indicated amino acids in each case. (iii) The structures of two chemically distinct versions of the AI-2 signaling compound have been determined. The AI-2 product of *V. harveyi* is a furanosyl borate diester (S-THMF-borate) while the product in *S. typhimurium* lacks boron and is referred to as R-THMF. (b) Two different types of autoinducer mimics. (iv) The first is an AHL mimic produced by *D. pulchra* and is a halogenated furanone. Note that this compound is structurally related to AHLs; however, it is apparent that not all AHL mimics closely resemble AHLs. (v) The mammalian hormones epinephrine and norepinephrine are derivatives of tyrosine and appear to function as mimics of a signaling compound related to AI-2 that has been named AI-3. The structure of AI-3 is currently unknown.

number. However, an alternative hypothesis has also been proposed in which autoinducer secretion functions as an indicator of the immediate cell environment in a process referred to as diffusion sensing [7]. Regardless of the exact function of QS systems, the molecular mechanisms underlying their functions have been extensively examined and characterized. As the focus of this review is on AHL-based QS systems, the reader is direc-

ted to recent reviews for details on peptide and AI-2-based systems [4,8].

2.1. AHL-based quorum sensing

The AHLs utilized by many gram-negative bacteria consist of a homoserine lactone ring joined to a fatty acid side chain that can vary in the number of carbons and modifications on the third carbon [1]. Upon entering a target bacterial cell, the AHLs bind to members of the LuxR family of helix-turn-helix transcription factors and modulate their activity. Selective recognition of AHLs by their cognate receptors contributes to the specificity of the signaling mechanism and also allows cross species communication between bacteria that synthesize similar or identical autoinducer molecules.

The mechanism of QS has been extensively characterized in several pathogenic bacteria including *P. aeruginosa*. This bacterium causes catastrophic infections in immunocompromised hosts, such as individuals with cystic fibrosis, cancer and severe burns. *P. aeruginosa* has at least two QS systems, named the Las and Rhl systems. The *LasI* and *RhlI* proteins catalyze the synthesis of *N*-3-oxododecanoyl homoserine lactone (3OC₁₂-HSL), also referred to as *Pseudomonas* autoinducer 1 (PAI-1), and *N*-butyryl-homoserine lactone (C₄-HSL, PAI-2), respectively. These autoinducers bind to and activate two LuxR homologues named LasR and RhlR. Many of the QS controlled genes in *P. aeruginosa* encode virulence factors and mutant strains with defective QS systems exhibit significantly lowered virulence in several different animal models [9,10].

2.2. Effective concentrations of AHLs produced in vivo

We will describe below evidence demonstrating that AHLs affect gene expression and other responses in host cells. However, as these effects have been observed at a range of experimental concentrations (see Table 1), it is important to consider the concentrations of AHLs that host cells are likely to encounter. In standard *P. aeruginosa* cultures, 3OC₁₂-HSL concentrations have been measured in the range of 2–10 μM [11,12]. These relatively low concentrations were measured under standard laboratory conditions (shaking at 37 °C in culture medium), which may not accurately reflect conditions encountered by host cells in vivo (e.g., exposure to biofilms, see below). Furthermore, the bioassays that have primarily been used to estimate autoinducer concentrations may suffer from a lack of sensitivity. In the standard autoinducer bioassay supernatant samples from AHL-producing cultures are removed and added to a new culture of a growing ‘indicator’ strain [11]. The ‘indicator’ strain, which is often another species of bacteria, is then grown for several hours, typically overnight, processed and assessed for β-galactosidase

Table 1
Documented cases of interkingdom signaling by bacterial acyl homoserine lactones

Autoinducer	Effective concentration	Target	Effect	Reference
<i>Plants</i>				
3OC ₁₂ -HSL	0.01–2 µM	<i>Medicago trunculata</i> (legume) seedlings	Differential regulation of >150 proteins	[33]
3OC _{16:1} -HSL				
C ₆ -HSL	ND	<i>Enteromorpha</i> (seaweed)	Enhanced zoospore settlement on bacterial biofilms	[73]
3OHC ₆ -HSL				
3OC ₁₀ -HSL				
<i>Animals</i>				
3OC ₁₂ -HSL	100 µM	16HBE (human bronchial epithelium) and L828 fibroblasts	Transcriptional activation of IL-8, activation of ERK1/2, NFκB and AP-2	[12]
3OC ₁₂ -HSL	100 µM	L828 fibroblasts	Transcriptional activation of Cox-2 and mPGES	[21]
3OC ₁₂ -HSL	0.3–0.5 pM	Human cystic fibrosis tracheal gland serous cell line (CF-KM4)	Inhibition of nucleotide receptor (P2Y2 and P2Y4) mRNA levels	[30]
3OC ₈ -HSL				
C ₈ -HSL	5–10 µM			
C ₄ -HSL				
3OC ₁₂ -HSL	0.1–100 µM	Murine and human primary cells	Inhibition of lymphocyte proliferation and of TNFα and IL-12 production by LPS-stimulated peritoneal macrophages. Modulation of antibody production by spleen cells at low or high concentrations, respectively	[22]
3OC ₁₂ -HSL	1–30 µM	Primary porcine arterial smooth muscle cell	Vasorelaxation	[28]
3OC ₁₂ -HSL	100 µM	Breast cancer cell lines	Induction of apoptosis, inhibition of STAT3 and partial inhibition of Akt/PKB	[24]
3OC ₁₂ -HSL	50 µM	Bone marrow derived macrophages and macrophage cell lines	Acceleration of apoptosis	[23]
3OC ₁₂ -HSL	1–100 µM	NCI-H292	Induction of MUC5AC	[31]

production. It is possible that significant degradation of autoinducers could occur between their initial synthesis, and their detection in the indicator strain, thereby yielding artificially low concentration estimates. In addition, specific or spontaneous degradation of purified or synthesized AHLs could also be a concern. Many studies examining the effects of AHLs on mammalian cells use synthetic autoinducers. AHLs are labile biological compounds that are subject to degradation; therefore great care must be exercised in handling and using synthetic autoinducers in these experiments. This includes not only an initial analysis of autoinducer purity by HPLC or NMR, but periodic re-analysis to examine the rate of breakdown and the identity of potential breakdown products. This is particularly important in light of a recent report suggesting that AHL degradation products may have activities independent of the parent AHL [13]. Thus, it will be crucial in the future to determine whether any observed effects could be attributed to an AHL or to a breakdown product. Finally, compounding factors in cell culture and in vivo systems may influence the effective concentration of AHLs in both positive and negative ways. There is evidence that host cells and bacterial cells produce “quorum quenching” enzymes that degrade autoinducers (see Section 4.4 of this review). For example, lactonases or other enzymes in cell culture

media could rapidly degrade any added AHL and thereby reduce the effective concentration. Conversely, it is also possible that the effects of bacterial autoinducers on host cells in vivo might be magnified through synergy with other bacterial or host effector molecules. For example, it has been proposed that the QS-controlled surfactant rhamnolipid that is produced by *P. aeruginosa* may act to solubilize autoinducers and enhance their effects [14].

Some of the earliest evidence that host cells could be exposed to high levels of autoinducers came from studies on the symbiotic relationship between *V. fischeri* and the sepiolod squid *Euprymna scolopes* [15]. *V. fischeri* resides within the light organ of *E. scolopes* and cell numbers can reach densities up to 10¹⁰ cells per ml. Consequently, autoinducer concentrations within the light organs were as much as 200 times higher than that observed in culture. Furthermore, host cells were directly exposed to autoinducers, which were not only found directly around the bacteria, but had also penetrated into underlying layers of the squid’s epithelia [15]. While the local concentrations of autoinducer that are achieved by pathogenic bacteria like *P. aeruginosa* in vivo are not known, *P. aeruginosa* is found at high concentrations (10⁸ cfu/ml) in the sputum of cystic fibrosis patients, and biologically active *P. aeruginosa* autoinducers have

been purified from sputum [16]. It is likely that the highest concentrations of autoinducers would be encountered by cells in the local vicinity of a biofilm and 3OC₁₂-HSL concentrations up to 600 μM have been measured in biofilm cultures grown in vitro [17]. Thus effects of autoinducers on host cells at concentrations up to this value may be considered potentially significant.

2.3. AHL-dependent modulation of gene expression in animal cells

The concept that QS signaling molecules might directly affect gene expression in human and other eukaryotic cells emerged from studies on the consequences of *P. aeruginosa* infection on chemokine expression in lungs of a CF model mouse strain. This and other examples are summarized in Table 1. The extensive tissue damage that is a hallmark of *P. aeruginosa*-infected lungs appears to be due to high level expression of the neutrophil chemokine interleukin-8 (IL-8), leading to extensive migration of neutrophils to the site of infection. Unrestrained activation of these neutrophils can lead to tissue damage and contribute to pulmonary disease in CF patients [18]. Initial investigations indicated that a small, heat-stable factor produced by *P. aeruginosa* was capable of inducing high level expression of IL-8 in primary and transformed bronchial epithelial cells and in monocytes [19]. The heat-stable factor was subsequently identified as 3OC₁₂-HSL [18] and the potential clinical relevance of this observation was highlighted by the detection of both *P. aeruginosa* autoinducers in the sputum of CF patients [16,20].

The identification of IL-8 as a 3OC₁₂-HSL-responsive gene suggested that this autoinducer molecule might participate in the infection process by modulating the host immune response. Further evidence to support this hypothesis emerged from subsequent identification of additional genes regulated by 3OC₁₂-HSL, including those encoding cyclooxygenase-2 (Cox-2) and the microsomal form of prostaglandin E synthase, leading to increased production of prostaglandin E₂ (PGE₂) [21]. In a separate set of experiments, Telford et al. [22] showed that 3OC₁₂-HSL inhibited the production of TNFα and IL-12 in lipopolysaccharide (LPS)-stimulated murine peritoneal macrophages, thereby favoring an anti-inflammatory Th-2 immune response as opposed to a Th-1 response. Many of these latter effects were observed at AHL concentrations lower than those used in the studies described above and it would appear that these host responses would benefit the bacterial pathogen rather than protecting the host.

3OC₁₂-HSL was recently found to modulate the expression of two additional chemokine genes in macrophages [23]. Interestingly, the effects on macrophage inhibitory protein (MIP)-2 and monocyte chemoattractant protein (MCP)-1 expression were opposite at lower

concentrations, with MIP-2 levels increasing and MCP-1 levels decreasing at concentrations up to 10 μM. At higher concentrations of 3OC₁₂-HSL, MIP-2 levels also decreased and this decrease was due to the induction of cell death. 3OC₁₂-HSL induced cell death only in bone marrow derived macrophages and in macrophage/monocyte cell lines but had no significant effect on the viability of cell lines of epithelial origin. In a related study, 3OC₁₂-HSL also blocked proliferation and induced apoptosis in human breast cancer cell lines [24].

From these studies it appears that 3OC₁₂-HSL can elicit both pro- and anti-inflammatory responses in host cells. These apparently contradictory results may result from differences in experimental procedures (i.e., exposure to 3OC₁₂-HSL in vitro or in vivo), the identity of the responding cell type, or perhaps the genetic background of the mouse strain being studied [25]. However, it has been argued that the disparity between inhibitory immunomodulatory effects observed at lower AHL concentrations [22] and stimulatory immunomodulatory effects at higher AHL concentrations [12,21,26] may be explained as part of a continuum of responses at different stages during the infection process that ultimately benefit the bacteria [27]. Specifically, inhibition of immune responses and inflammation at low AHL concentrations (and presumably low bacterial cell numbers) would prevent clearance of invading bacteria at early stages of infection, while enhancement of inflammation at high AHL concentrations (and thus high cell numbers) would potentially enable the more rapid dissemination of bacteria from the site of infection (Fig. 2). Interestingly, AHLs have also been found to influence smooth muscle contraction in blood vessels [28,29]. These vasodilatory effects could alter blood flow rates in the vicinity of an infection and further enhance bacterial dissemination.

AHLs can also affect gene expression in an airway epithelial cell line model of cystic fibrosis, which lacks functional CF transmembrane conductance receptor (CFTR) protein [30]. Four different AHLs were tested and 3OC₁₂-HSL inhibited expression of two nucleotide receptors, P2Y₂ and P2Y₄, at picomolar concentrations in the CF cell line, but not in a normal cell line. C₄-HSL had a similar effect when applied at significantly higher concentrations. These inhibitory effects were reversible either by adenoviral-mediated expression of a wild-type CFTR protein, or by treatment of the cells with ibuprofen or nordihydroguaiaretic acid but not dexamethasone or indomethacin. The differential ability of these anti-inflammatory agents to block AHL-mediated repression of P2Y expression may implicate leukotrienes in this process; however, this has not yet been verified experimentally. An additional possible connection between 3OC₁₂-HSL signaling and cystic fibrosis emerged from the recent demonstration that 3OC₁₂-HSL can induce expression of the MUC5AC mucin gene in the human lung NCI-H292 epithelial cell line [31].

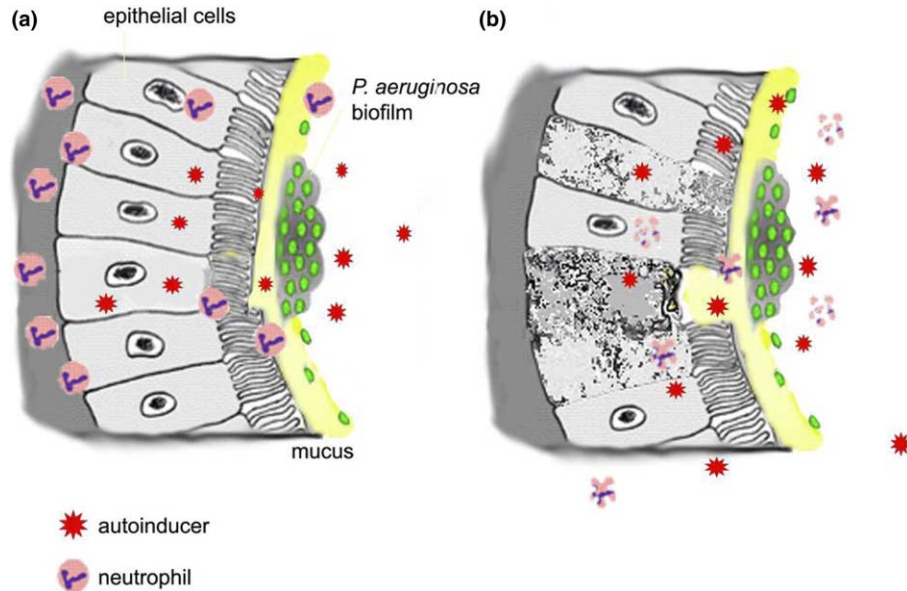


Fig. 2. A model for the effects of high concentrations of 3OC₁₂-HSL on host cells. (a) A *P. aeruginosa* biofilm has formed adjacent to an epithelial cell layer in the lung. 3OC₁₂-HSL is released from the cells within the biofilm yielding high local concentrations. The AHLs penetrate the epithelial cell layer, activating chemokine gene expression and initiating a local inflammatory response (indicated by the recruitment of neutrophils). (b) High concentrations of 3OC₁₂-HSL activate apoptotic responses in neutrophils while epithelial cells in the vicinity of the biofilm are destroyed due to an unrestrained local inflammatory response. A major outcome of these effects would be extensive permanent damage to lung tissue and/or systemic spread of the bacteria.

2.4. AHL-dependent modulation of gene expression in plant cells

Some strains of *P. aeruginosa* are also plant pathogens [32], as are other pseudomonads, and research on the model legume *Medicago trunculata* suggests that AHLs also influence plant gene expression. In the first report of a proteomic study in this area, roots of 3-day-old *M. trunculata* seedlings were exposed to either 3OC₁₂-HSL or 3OC_{16:1}-HSL, a QS signaling molecule from the nitrogen-fixing symbiont *Sinorhizobium melliloti* [33]. Protein expression was analyzed by two-dimensional gel electrophoresis and differentially-expressed proteins were identified by mass spectrometry. The abundance of over 150 proteins was altered by AHL exposure and the specific subset of proteins that displayed altered levels was dependent on the identity and concentration of the AHL and the time of exposure. As in mammalian cells, different responses were observed upon treatment with varying concentrations of AHL. For example, out of a group of 60 proteins whose level of accumulation increased significantly in response to 50 μM 3OC₁₂-HSL, the abundance of 53 decreased significantly in samples exposed to lower concentration of this AHL. These differential responses to varying concentrations of AHL therefore resemble the effects seen in mammalian cells and suggest that the specific plant cell response to AHL depends on the bacteria cell number. In addition, 3OC₁₂-HSL activated expression of transgenic reporter genes driven by promoters derived from

genes related to some of those identified in the proteomic analysis, indicating that the observed changes are likely to be due to direct effects of 3OC₁₂-HSL on transcription. The identified proteins were assigned to several functional classes, including 23% related to plant defense and stress response, 14% potentially involved in protein degradation or processing and 37% to energetics and metabolism. Importantly, the abundance of approximately 6% of the resolved proteins in this study changed in response to AHL [33]. Interestingly, microarray analyses revealed that between 5% and 10% of *P. aeruginosa* genes are QS-regulated [34,35] indicating that the magnitude of the response of eukaryotic cells to AHL may approach that observed in bacteria. These studies will undoubtedly be invaluable in elucidating the mechanism by which plant cells respond to bacterial autoinducers and potentially in identifying putative eukaryotic AHL receptor proteins (see below).

To conclude this section, it is becoming apparent that eukaryotic cells are capable of actively responding to bacteria in their immediate environment by detecting and acting upon the presence of autoinducer molecules secreted by invading bacteria. Most of these data have thus far focused on a limited number of AHL molecules, most notably the 3OC₁₂-HSL molecule secreted by *P. aeruginosa*. This focus presumably reflects the early recognition that QS, and its associated factors, is crucial for many aspects of *P. aeruginosa* pathogenesis. In several of the studies described above, the activity of 3OC₁₂-HSL was compared to the other major AHL produced

by *P. aeruginosa*, C₄-HSL. In most cases, C₄-HSL did not elicit any significant effects when compared to 3OC₁₂-HSL. Furthermore, a systematic analysis of the immunomodulatory activities of a variety of synthetic analogues of 3OC₁₂-HSL revealed that both the length and modifications of the acyl chain were critical determinants of activity with optimal activity achieved with AHLs containing 11–13 carbon side chains and either a 3-oxo or 3-hydroxyl modification [36]. Of course, this study only examined a limited set of AHL effects on eukaryotic cells and future studies on the effects of different AHLs on eukaryotic cell function may uncover additional specific activities. However, the apparent specificity of action of 3OC₁₂-HSL and its close relatives implies the existence of specific receptor proteins in eukaryotic cells that mediate its effects on gene expression. Although specific eukaryotic AHL receptor proteins have not yet been identified, the analysis of signaling pathways in eukaryotic cells modulated by 3OC₁₂-HSL has provided the first clues as to their identity.

3. Molecular mechanisms of AHL action in eukaryotic cells

In theory, there are at least three not-necessarily exclusive mechanisms by which AHLs could elicit effects in eukaryotic cells. Based on their hydrophobic structures and their ability to traverse bacterial cell membranes, it is possible that AHLs freely diffuse into mammalian cells and bind to intracellular receptor proteins. This mechanism of action would be comparable to the primary mechanism of action of steroid hormones and other lipid-based hormones, such as retinoic acid, that function as ligands for intracellular receptors. These receptors are transcription factors that belong to the nuclear hormone superfamily of zinc finger transcription factors [37]. Three recent studies have clearly shown that AHLs of different structures can enter eukaryotic cells and retain functionality in this alien environment [38–40]. These studies used similar strategies that involved the conversion of LuxR-type proteins from either *Agrobacterium tumefaciens* (TraR) or *P. aeruginosa* (LasR and RhIR) into transcription factors that could function in mammalian cells in culture and the demonstration that they retained responsiveness to their cognate autoinducers. A second potential mechanism of action for AHLs in eukaryotic cells is as modulators of intracellular biochemical reactions. In this regard, AHLs could either function to regulate the activity of enzymes, or potentially act as substrates within biochemical pathways. One possible outcome of the latter potential function might be the altered production of a hormone with gene regulatory properties. This potential function may be assessed by following the fate of radiolabeled AHL

components within specific cell types. The third potential mechanism of AHL action is as extracellular ligands for membrane-associated receptor proteins. At present, there is no direct evidence in support of this hypothesis although it is noteworthy that non-genomic effects of certain steroid hormones, such as estradiol and progesterone, can be elicited through membrane-bound receptors in addition to their better known nuclear receptors [41]. In addition, other lipid-based mammalian signaling compounds such as prostaglandins elicit their effects through both membrane associated and intracellular receptors [42].

3.1. Targets of AHL action: transcription factors and protein kinases

The goal of identifying bona fide AHL receptors in mammalian cells is still at the early stages, although some components of the signaling pathways used by AHLs in certain cells have been described. The pathways implicated thus far are schematically summarized in Fig. 3. First, analysis of promoter regions required for IL-8 responsiveness to 3OC₁₂-HSL in a human bronchial epithelial cell line identified NFκB and AP-2 as two potential transcription factors involved in mediating AHL activity [12]. The role of NFκB in this regulatory pathway was supported both by mutagenesis of a specific NFκB binding site within the IL-8 promoter and by electrophoretic mobility shift assays, which detected NFκB binding activity only in extracts from cells exposed to 3OC₁₂-HSL. NFκB was subsequently also implicated in the regulation of Cox-2 by 3OC₁₂-HSL [21]. NFκB is a multifunctional transcription factor of the Rel family and functions as a key regulator of inflammation, apoptosis and development [43]. NFκB exists in a latent form in the cytoplasm of unstimulated cells, where it is sequestered by the IκB proteins. Stimulation by cytokines, LPS and presumably by 3OC₁₂-HSL results in phosphorylation, ubiquitylation and degradation of IκB, freeing NFκB to migrate to the nucleus where it can regulate target genes. NFκB generally functions as a dimer and the form implicated in IL-8 transcriptional regulation is a heterodimer of p50 and p65 subunits. Two members of the MAP kinase family of serine/threonine kinases, ERK-1 and ERK-2, were also activated by 3OC₁₂-HSL in lung epithelial cells and NFκB activation was blocked by pharmacologic inhibition of MAP kinase activity. These data thus suggest that the ERKs are upstream mediators of 3OC₁₂-HSL-dependent NFκB activation in these cells. A similar pathway has been implicated in the induction of the MUC5AC gene in NCI-H292 cells [31].

The conclusion that AP-2 is a 3OC₁₂-HSL-responsive regulator of IL-8 transcription is based on the fact that AP-2 DNA binding activity was induced in 3OC₁₂-HSL treated cells and that deletion of a large promoter region

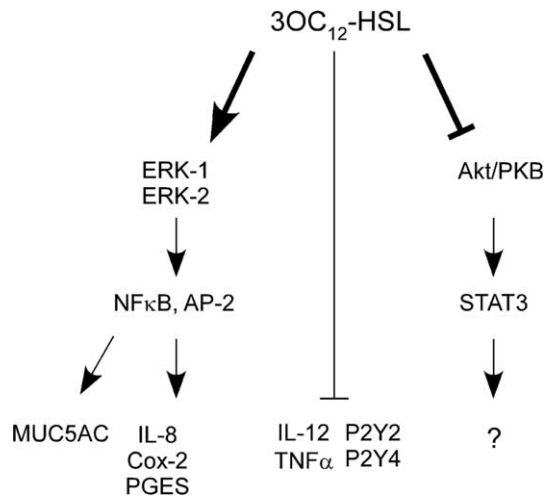


Fig. 3. Signal transduction pathways implicated in $3OC_{12}$ -HSL signaling. Pathways and genes activated or inhibited (represented with an arrowhead or perpendicular line, respectively) by higher concentrations of $3OC_{12}$ -HSL (up to $100\ \mu\text{M}$) are shown to the left and right, respectively. Genes inhibited by low concentrations (0.3 – $100\ \text{pM}$) of $3OC_{12}$ -HSL are shown in the center of this diagram. Key: ERK, extracellular regulated kinase; IL-8, 12, interleukin 8 or 12; Cox-2, cyclooxygenase 2; PGES, prostaglandin E synthase; MUC5AC, mucin 5AC gene; TNF α , tumor necrosis factor alpha; P2Y2, P2Y4, purine nucleotide receptors; Akt/PKB, protein kinase B; STAT3, signal transducer and activator of transcription 3.

encompassing an AP-2 binding site reduced $3OC_{12}$ -HSL-responsiveness. AP-2 had not previously been implicated in IL-8 gene regulation although it is involved in the regulation of other cytokine genes. Additional studies, including the identification of $3OC_{12}$ -HSL-responsive regions of additional gene promoters will undoubtedly aid in establishing signal transduction pathways mediating AHL action in lung epithelial cells.

A different signaling pathway was implicated as mediating the effects of $3OC_{12}$ -HSL on proliferation and apoptosis in breast cancer cells [24]. In this case, AHL treatment led to inhibition of the activity of the signal transducer and activator of transcription (STAT) 3 transcription factor. ERK activity was not affected in these cells although partial inhibition of the Akt/PKB signaling pathway was observed. Thus it is possible that the effects of $3OC_{12}$ -HSL are cell specific and future studies will test this hypothesis. In addition, as these studies only focused on $3OC_{12}$ -HSL, additional studies will be necessary to determine whether other AHLs utilize similar or unique signaling pathways.

3.2. Structural correlations from *LuxR* homologues

Despite these advances in our understanding of the molecular consequences of AHL action on eukaryotic cells, the identification of a specific eukaryotic AHL receptor remains elusive. None of the transcription factors and kinases mentioned above is likely to be directly regulated by AHL binding. However, elucidation of the

three dimensional structure of the AHL binding domain of the TraR protein from *A. tumefaciens* by two groups has provided some clues about structural features that might be found in this putative receptor [44,45]. Both studies showed that the AHL ($3OC_8$ -HSL) was buried within a cleft in the N-terminal domain of TraR, and that the structure of this domain resembled that of GAF or PAS domains [46]. GAF domains (named for their presence in cyclic GMP (cGMP)) regulated cyclic nucleotide phosphodiesterases (PDEs), a subset of adenylyl cyclases and the bacterial transcription factor (FhlA) and PAS domains (named for period, ARNT and Sim proteins) [47] are structurally related and are found in several hundred signaling proteins and transcription factors in all three kingdoms of life. These domains function as small molecule sensors as, for example, in a subset of PDEs where GAF domains function as high affinity cGMP binding sites that regulate enzymatic activity [48,49]. The PAS domain has been proposed to be a signature motif within proteins that sense environmental change and, for example, is widely used in transducing light and redox-dependent signals [50]. In addition, PAS domains are found in the aryl hydrocarbon receptor and hypoxia inducible factors, two transcription factors involved in mediating transcriptional responses to planar aromatic hydrocarbons and oxygen, respectively [46]. Thus, it is certainly possible that AHL receptors might be found amongst the GAF or PAS domain containing proteins in eukaryotic cells. Ultimately, the combined use of biochemical pathway mapping strategies, identification of AHL-responsive genes and molecular modeling approaches will likely be used to identify these receptor proteins.

4. Talking back: the biology of AHL mimics

Several recent studies have shown that this communication pathway is not one-way and that eukaryotic cells produce chemical compounds that can interfere with QS systems in bacteria by acting as agonists or antagonists of AHL signaling pathways. Although in several cases these eukaryotic products do not appear to be structurally related to AHLs, they are generally referred to as AHL mimics in reference to their functional status. The consequences of interacting, or interfering, with QS systems are reasonably obvious. For example, in pathogenic bacteria where QS systems are designed to prevent activation of virulence gene expression at low cell density, the ability to prematurely activate expression of these genes would force the bacterium to reveal its presence at a time during the infection process when the host immune system could effectively prevent its establishment. In the case of pathogenic bacteria this mode of interaction may not actually represent true communication and it has been commonly referred to

as signal interference [51]. Alternatively, in symbiotic or commensal relationships, direct interactions between gene regulatory networks in the prokaryotic and eukaryotic partners may permit the coordination of complementary developmental programs. The commensal relationship between intestinal cells and the enteric bacteria of the mammalian gut is just one prime candidate where such an interaction could be of extreme benefit to both partners. For example, exposure of germ-free mice to just one species of its normal flora (*Bacteroides thetaiotaomicron*) resulted in multiple changes in gene expression in intestinal epithelial cells [52]. Although this situation may involve bidirectional communication between host cells and bacteria, AHLs have not yet been implicated in this relationship.

4.1. Furanones

The first AHL mimic was identified in the marine red alga, *Delisea pulchra*, which produces a halogenated furanone that interferes with QS systems in marine bacterial species (Fig. 1). Specifically, the swarming motility, but not the growth, of both *Serratia liquefaciens* [53] and *Proteus mirabilis* [54] was inhibited in the presence of furanones. Furthermore, it was shown that the furanones interfered with communication between bacterial species during colonization of the algal thalli through competition with cognate signal molecules [55]. The halogenated furanones function by displacing the normal ligand from LuxR and promoting its rapid degradation [56,57]. Halogenated furanones of *D. pulchra* also strongly antagonized the AHL produced by the black tiger prawn pathogen, *V. harveyi*, inhibiting both luminescence and virulence [58]. Furanones also behaved as an AHL mimic in a non-marine species of bacteria, *P. aeruginosa*. In this model, a synthetic halogenated furanone was reported to affect the architecture of *P. aeruginosa* biofilms and enhance bacterial detachment leading to reduced biomass [59]. AHL mimics have attracted significant interest as possible alternatives for antibiotics and initial studies demonstrated that a synthetic halogenated furanone enhanced antibiotic sensitivity in *P. aeruginosa* biofilms and decreased virulence in a mouse pulmonary infection model [60].

4.2. Diketopiperazines

Diketopiperazines (DKPs) are a family of cyclic dipeptides that have been isolated in the supernatant of numerous cultures of bacterial species, including *P. aeruginosa*, *P. mirabilis*, *Citrobacter freundii*, and *Enterobacter agglomerans* [61], as well as from protein hydrolysates and fermentation broths from yeast, lichen, and fungi [62]. Interestingly, some DKPs share structural similarity to endogenous signaling peptides, such as thyrotropin-releasing hormone [62], and one DKP (cyclo-

(L-His-L-Pro)) has been identified in mammalian tissues. Holden et al. reported that DKPs can modulate quorum-dependent phenotypes in several different species of bacteria by acting as AHL antagonists in some LuxR-based QS systems and as agonists in others [61]. Differential recognition of various DKPs by different LuxR homologues suggests that these AHL mimics may play a role in both intra- and interspecies QS regulation.

4.3. Mimics in higher plant species

Many higher plant species also secrete substances that mimic AHL signals and affect QS behaviors in bacteria. As in animal cells, these mimics may serve a defensive function by preventing the production of virulence factors in pathogenic organisms. Interestingly, plant-derived AHL mimics include substances that both stimulate and inhibit bacterial QS systems. For instance, components of pea seedling exudates inhibited AHL-induced violacein synthesis in *Chromobacterium violaceum*, induced swarming activity in *S. liquefaciens* MG44, which is defective in AHL synthesis, and induced luminescence in *E. coli* reporters containing plasmids encoding either LuxR from *V. fischeri*, AhvR from *Aeromonas hydrophila*, or LasR from *P. aeruginosa* [63]. In addition, extracts from rice, soybean, tomato, crown vetch, and *Medicago truncatula* all contain AHL mimics [33,63,64]. The unicellular green alga *Chlamydomonas reinhardtii* also produces substances that interfere with bacterial QS systems [65]. Interestingly, a subset of compounds fractionated from *C. reinhardtii* extracts using an ethyl acetate-based extraction procedure similar to that used to purify AHLs from bacterial culture supernatants stimulated the activity of LuxR-type proteins. One such compound, selected as a LasR activator, elicited similar, although not identical, changes in gene expression in *S. melliloti* as bonafide AHLs, suggesting that these ethyl acetate-extractable compounds are structurally related to AHLs. A second set of compounds isolated from *C. reinhardtii* that functioned as QS inhibitors did not co-fractionate with these stimulatory activities, suggesting that they are likely to be structurally distinct from AHLs. Although the exact structures of these compounds have not yet been determined, the genetic amenability and the availability of the complete genome sequence of *C. reinhardtii* should permit the rapid identification of genes associated with these responses.

4.4. Enzymatic interference with QS

An alternative mechanism for interfering with QS signaling has emerged from the discovery of a specific activity associated with several human and non-human cell types that inactivates subsets of AHLs [66]. Initial

studies showed that 3OC₁₂-HSL concentrations decreased rapidly after exposure to the apical surface of differentiated airway epithelial cells while C₄-HSL concentrations only decreased slightly. The specificity of this inactivation process was further documented using an expanded set of AHLs that differed in the length and modification of the acyl chain. In addition to 3OC₁₂-HSL, C₆-HSL was efficiently inactivated while 3OC₆-HSL and C₁₂-HSL were not. The pattern of AHL sensitivity to inactivation did not correlate with length or modification of the acyl side chain and the inactivating activity was membrane associated and heat-labile. These data suggest that the AHL inactivation is due to a cell-associated enzymatic activity. Previous studies in transgenic plants had demonstrated that overexpression of AHL degrading enzymes provided protection against bacterial infections [67] and these results indicate that some mammalian cell types possess an innate ability to disrupt bacterial infections. Deficiencies in such systems may predispose individuals to bacterial infections, such as in chronic lung *P. aeruginosa* infections in cystic fibrosis patients. Furthermore, the cell specificity of the inactivation process may partially explain the disparate immunomodulatory effects of 3OC₁₂-HSL described earlier.

4.5. Hormonal mimicry in the LuxS/AI-2 pathway

A direct demonstration of hormonal intercommunication between bacteria and mammals has emerged from studies on a QS system that is not based on AHLs. As mentioned above, there are at least two other QS systems in bacteria one of which is the LuxS/AI-2 signaling system [4,8]. LuxS catalyzes the formation of (4S)-4,5-dihydroxy-2,3-pentanedione (DPD) from *S*-ribosylhomocysteine and at least two chemically distinct signaling compounds derived from DPD have now been identified (Fig. 1). The LuxS/AI-2 signaling system has been implicated in controlling many different genes and phenotypes in different bacterial species, including enterohemorrhagic *E. coli* (EHEC) [4]. EHEC colonize the large bowel and are responsible for outbreaks of bloody diarrhea and hemolytic uremic syndrome. EHEC form lesions named attaching and effacing (AE) on intestinal epithelial cells and the formation of the AE lesion requires a set of genes on the locus of enterocyte effacement (LEE) pathogenicity island. Expression of several of the LEE genes is under QS control and their expression was diminished in an EHEC *luxS* mutant strain [68]. Surprisingly, the mutant strain retained the ability to form AE lesions that were indistinguishable from those produced by the wild type strain when grown on cultured HeLa epithelial cells [68], suggesting that a component of the culture system could substitute for the QS signaling molecule. This component was subsequently identified as either of the catecholamines epi-

nephrine (Epi) or norepinephrine (NE), two closely related eukaryotic hormones derived from the amino acid tyrosine (Fig. 1). These hormones were present in the fetal bovine serum used to supplement the HeLa growth medium and both are present within the gastrointestinal tract [69]. Epi and NE are structurally quite different from AI-2 and further analysis revealed that the two mammalian hormones were actually mimicking a novel activity that is also a product of the LuxS pathway and has been named AI-3. Although AI-3 has not yet been completely characterized, it displayed structural properties that indicate that it is distinct from either of the two forms of AI-2 shown in Fig. 1, Epi or NE. The physiological significance of cross-communication between a bacterial autoinducer and two mammalian hormones is unclear at present, however, the authors suggest that it may control normal interactions between intestinal epithelial cells and commensal bacteria within the normal gut flora [68], several of which contain the LuxS QS system [4]. The responsiveness of EHEC to these signals may be an unfortunate consequence of a pathogenic bacterium adapting the LuxS system for regulation of virulence gene expression.

5. Conclusions and perspectives

Returning to the quotation from the Stoic philosopher Epictetus that began this review, we are beginning to understand how AHL-based QS systems form the basis of a molecular language shared by bacteria and eukaryotic cells. AHLs produced by bacteria can apparently directly interact with as yet unidentified eukaryotic proteins and modulate gene expression in the host cell. Likewise, eukaryotic cells produce compounds that interact with components of the bacterial QS systems, thereby demonstrating that information can pass in both directions. The major questions that remain to be answered lie primarily in understanding how the information is interpreted on either side of the eukaryote/prokaryote divide. Most of the examples cited in this review have highlighted interactions between pathogenic bacterial species and their hosts. In these cases, the primary goal of signal exchange appears to involve the manipulation of the target cell for the benefit of the signal producer. For example, the ultimate goal of pathogenic bacteria is to establish a productive infection in the host and thus AHLs appear to be used by these organisms to prevent the host immune system from mounting an effective defense. Similarly, the primary function of AHL mimics may be to interfere with the normal functioning of QS systems, which in pathogenic bacteria are often critical regulators of virulence gene expression. Thus these interactions do not resemble true communication systems, involving information exchange for the mutual benefit of both partners. Instead,

the AHLs and AHL mimics are employed in processes of chemical manipulation for the benefit of only one partner. This raises the important question of whether bacteria and eukaryotes truly communicate as equal partners using AHLs-based systems. There are several well-characterized scenarios where bacteria and their eukaryotic hosts coexist in symbiotic or commensal relationships, one of which, the interaction between mammalian intestinal cells and their normal flora has already been mentioned. Other examples include the colonization of the intestines of the nematode *Steinernema carpocapsae* by *Xenorhabdus nematophila* bacteria [70,71] and the colonization of the light organ of the squid *E. scolopes* by *V. fischeri* [72]. Current efforts to test whether AHLs are directly involved in the establishment of these mutually beneficial relationships will determine whether true interkingdom communication occurs or whether chemical manipulation is the primary mode of action of these signaling compounds.

Acknowledgements

The Rumbaugh and Williams laboratories are supported by grants from the South Plains Foundation, The CH Foundation, Texas Tech University Health Sciences Center, the American Lung Association and the American Heart Association. We also wish to thank Abdul Hamood, Jane Colmer-Hamood, Elmus Beale and Brandt Schneider for helpful comments on the manuscript.

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