Perception and Degradation of *N*-Acyl Homoserine Lactone Quorum Sensing Signals by Mammalian and Plant Cells

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1. Introduction

Cell-to-cell signaling via small chemical signals is an ancient process shared by most living organisms. Whether these signals are produced by mammals, insects, plants, or bacteria and are called hormones, pheromones, or autoinducers, they convey vital information about the status of the cell and its extracellular environment. The discovery of bacterial cell-to-cell signaling, or quorum sensing (QS), revealed that single-celled prokaryotic organisms possess sophisticated methods for coordinating their behavior by secreting chemical or peptide signals and ushered in a new field of Microbiology.

The study of quorum sensing has mainly focused on identifying the components making up quorum sensing circuits in bacteria, and significant progress has been made in elucidating the structures of the chemical signals, identifying the receptors that detect these signals and affect QS-dependent gene expression, and mapping the signal transduction pathways involved. However, we are also beginning to understand that these bacterial quorum sensing signals (QSS) are perceived, responded to, and degraded by both eukaryotes and members of the host-associated microbial communities. Thus, the question arises, "How do host cells perceive QSS and what are the consequences of this cross-kingdom communication?"

QSS are structurally and functionally similar to some hormones produced by mammals and phytohoromones made by plants, and there is strong evidence to suggest that they

Max Teplitski is an associate professor in the Soil and Water Science Department at the Genetics Institute of the University of Florida. The Teplitski group focuses on defining the roles of chemical signals and nutritional cues in structuring and functions of host-associated bacterial communities. Mechanisms by which plant and animal hosts manipulate cell-to-cell signaling and population density-dependent gene regulation in the associated symbiotic and pathogenic bacteria are of special interest to his group. To address these questions, members of the Teplitski team investigate the role of quorum sensing and post-transcriptional regulation in the *Sinorhizobium*−*Medicago* symbiosis, functional genomics of the interactions between *Salmonella* and plant hosts, and the interspecies interactions within microbiota of the coral mucopolysaccharide layer.

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Ulrike Mathesius was born in Germany where she received her Dipl. Biol. (B.Sc. Hons.) from the Technical University of Darmstadt in 1995. Inspired by a year of research during her undergraduate studies at the Research School of Biological Sciences at the Australian National University (ANU) in Canberra, she moved down under. She received her Ph.D. from the ANU in 2000, which focused on the symbiosis between rhizobia and legumes. This was followed by postdoctoral research at the ANU in the area of plant proteomics until 2001. In 2002, she moved to the School of Biochemistry and Molecular Biology with a Post Doctoral Fellowship from the Australian Research Council to work on plant perception of bacterial signals. She currently holds an ARC Research Fellowship at the ANU. Her research focuses on the interaction of roots with symbiotic and parasitic soil microbes and tries to identify molecular mechanisms of plant responses to microbial signals.

Kendra Rumbaugh was born in New Mexico and received her B.S. in Microbiology from the University of Texas, El Paso, in 1996. During her undergraduate years, she worked as a research assistant in a plant physiology laboratory at the Texas A&M Research Extension Center in El Paso. She attended graduate school at the Texas Tech University Health Sciences Center (TTUHSC) in Lubbock, and her doctoral work focused on the role of quorum sensing in the pathogenesis of *Pseudomonas aeruginosa*. After receiving her Ph.D. in medical microbiology in 2001, she received a postdoctoral training fellowship from Cystic Fibrosis Research Inc. and moved to San Francisco to work in the Wiener-Kronish laboratory at UCSF. In 2002, she returned to TTUHSC and initiated her own studies investigating the role of interkingdom signaling in host/bacterial interactions. In 2006, she became an Assistant Professor in the Department of Surgery where she continues her research in quorum sensing and biofilm-related wound infections.

exert more than just "nonspecific" effects on these host cells (Table 1). Although a wide variety of effects have been reported and will be discussed below, the main class of bacterial signal that has been shown to mediate these effects is the acyl-homoserine lactones (AHLs) produced by Gram-negative bacteria. AHLs are the most prevalent and well-studied bacterial signals described thus far, although there are many other known QSS (for examples, see refs 1 and 2); the scope of this review is limited to the interaction of AHLs with plants and animals.

Despite the fact that AHLs were first discovered in bacterial symbionts of mollusks, a role for AHL-mediated QS is not universal in animal-associated microbiota. In mammals, members of commensal microbiota are not known for their ability to produce AHLs. For example, *Escherichia coli* (and its pathogenic relatives, *Shigella* spp, *Salmonella* spp., and *Klebsiella* spp) lack the AHL synthase genes and do not produce their own AHLs. Their genomes, however, encode a functional AHL receptor, SdiA.[3] Conversely, most Gram-negative bacterial pathogens of mammals produce AHLs and use them for controlling virulence genes.[4] Synthesis of AHLs and AHL mimics appears to be common in commensals and pathogens of invertebrates, while only pathogens of mammals use AHL-mediated QS. QS in plant-associated bacterial communities is even more nuanced, because pathogens, commensals, and symbionts produce and degrade AHLs, while plants detect and respond to bacterial AHLs and also produce and exude AHL-mimics. Mechanisms underlying these interactions and their ecological consequences are considered in this review.

AHLs were originally called autoinducers, because binding of AHLs to their cognate receptors led to a positive feedback loop, thus increasing concentrations of AHLs within a diffusion-limited environment occupied by the bacteria.[5] These chemical ligands transduce changes in bacterial transcription by altering the conformation of their cognate receptors, which are typically members of the LuxR family of transcriptional regulators. However, their activities may not be limited to bacterial receptors, because there are recent data suggesting that they may also act as ligands for some mammalian receptors.[6]

It is also clear that some eukaryotic hosts possess enzymes that degrade or inactivate AHL signals or produce their own soluble signals that can mimic AHLs. This indirectly suggests that hosts have adapted specific mechanisms for detecting AHLs in the extracellular environment, and that autoinducers may be "interkingdom signals" that allow bacteria to directly interact with the signaling processes of their eukaryotic hosts.[7] In this review, we will describe the various effects of AHL autoinducers on mammalian and plant cells, the mechanisms by which these signals are conveyed and transduced between kingdoms, and the strategies by which the host deals with them.

2. Perception of N-Acyl Homoserine Lactone QSS by the Mammalian Host

The mammalian microbiome is integral to the healthy physiology of the host. The cellular content of a healthy mammal is 90% bacterial and these bacteria contribute antipathogen protection and influence development of the GI tract and other organs.[8] However, these organisms exist in a delicate balance, and alterations to their population can be detrimental to individuals in diseased states.[8] Thus, if we consider the host-associated microbiome an organ system, just as the skin or nervous system, and take into account the sheer number of bacterial cells present, then we have to consider the potential influences that their vast array of soluble signaling molecules have on host cells.

Not surprisingly, the studies examining the effects of bacterial signaling compounds on mammalian cells have focused on their role in pathogenesis.[7] While interkingdom signaling may have as yet undiscovered roles in mediating relationships between host cells and commensals, most work

Table 1. Survey of Studies Examining the Effects of Bacterial Signals on Host Cells

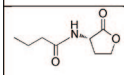
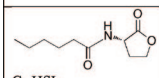
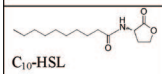
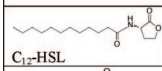
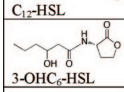
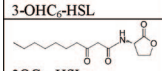
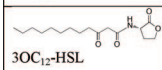
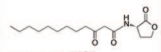
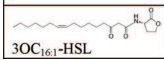
Bacterial Signal	Host-cell response reported	Effective concentration	Type of host cell studied	Proposed mechanism	Refs
 C_4-HSL	Inhibition of nucleotide receptor (P2Y2 and P2Y4) mRNA levels	5-10 μM	Human cystic fibrosis tracheal gland serous cell line (CF-KM4)	None	23
	Regulation of expression of several defense related genes; mediation of systemic resistance	10 μM	Tomato (*Lycopersicum esculentum*) leaves	Induction of salicylic acid and ethylene signaling pathways	44
	Root elongation	10 μM	*Arabidopsis thaliana* roots	Alteration of auxin and cytokinin concentration	45
 C_6-HSL	Enhanced settlement of *Enteromorpha (Ulva)* zoospores	50 μM	*Enteromorpha (Ulva)* zoospores	None	41
	Alteration of mimic compound exudation	2 nM	*Medicago truncatula* roots	None	43
	Regulation of expression of several defense related genes; mediation of systemic resistance	10 μM	Tomato (*Lycopersicum esculentum*) leaves	Induction of salicylic acid and ethylene signaling pathways	44
	Regulation of expression of multiple genes Root elongation	10 μM	*Arabidopsis thaliana* roots and shoots	Alteration of auxin and cytokinin concentration	45
 C_{10}-HSL	Increase of lateral root density and accelerated development. Stimulation of root hair development	>48μM	*Arabidopsis thaliana* roots	Inhibition of cell division in root meristem	46
 C_{12}-HSL	Stimulation of root hair development	>48μM	*Arabidopsis thaliana* roots	None	46
 3-OHC$_6$-HSL	Enhanced settlement of *Enteromorpha (Ulva)* zoospores	50 μM	*Enteromorpha (Ulva)* zoospores	None	41
 3OC$_{10}$-HSL	Enhanced settlement of *Enteromorpha* zoospores	50 μM	*Enteromorpha (Ulva)* zoospores	None	41
	Reduced zoospore swimming speed	25 and 125 μM	*Enteromorpha (Ulva)* zoospores	None	42
 3OC$_{12}$-HSL	Induction of IL-8 production	Unknown (supernatant)	Primary and a bronchial epithelial cell line (16-HBE) and monocytes	None	136
	Inhibition of lymphocyte proliferation, TNFα and IL-12 production after LPS-stimulation, and modulation of antibody production	0.1-100 μM	Murine peritoneal macrophages, primary murine spleen cells, human peripheral blood and mononuclear cells	None	29
	Inhibition of nucleotide receptor (P2Y2 and P2Y4) mRNA levels	0.3-0.5 pM	Human cystic fibrosis tracheal gland serous cell line CF-KM4	None	23
	Vasorelaxtion	1-30 μM	Primary porcine arterial smooth muscle cells	None	137
	Stimulation of IL-8 production	100 μM	Human lung fibroblasts (L828), human alveolar epithelial cell line A549, primary human foreskin fibroblasts and human bronchial epithelial cell line 16HBE	Activation of ERK1/2, NFκB and AP-2	25
	Stimulation of IL-1α, IL-6, MIP-2, MCP-1α, MIP-1β, IP-10, TCA-3, COX-2 and IFN-γ	1-100 μM	Mouse-whole dermis, primary mouse keratinocytes and T-cells	None	28
	Stimulation of CoX-2, PGE synthase and mPGE$_2$ production	100 μM	Primary human lung fibroblasts	Activation of NFκB	26
	Stimulation of MIP-2 and apoptosis	50 μM	Bone marrow-derived macrophages and macrophage cell lines, and murine peritoneal neutrophils	Elevation of caspase 3 and 8 activities (extrinsic pathway)	14
	Reduction in IFN-γ and IL-4 protein secretion	4-10 μM	Stimulated primary splenocytes	None	31
	Apoptosis	100 μM	Several breast cancer cell lines	Inhibition of STAT3 and partial inhibition of Akt/PKB	15
	Stimulation of mucin (MUC5AC) production	1-100 μM	NCI-H292 epithelial cells	(ERK) 1/2 and IκB phosphorylation	24
	Stimulation of phagocytic activity	100 μM	Primary human macrophage-differentiated monocytes	Activation of p38 MAPK pathway	27
	Apoptosis and stimulation of IL-6, KC, and COX-2, IL-8	10-100 μM	Murine embryonic fibroblast cell line NIH3T3, human umbilical cord vein endothelial cell line HUVE-12	Increases in cytosolic Ca^{2+} levels via ER IP$_3$ receptors (for apoptotic effects)	16

Table 1. Continued

Bacterial Signal	Host-cell response reported	Effective concentration	Type of host cell studied	Proposed mechanism	Refs
 3OC12-HSL-HSL	Disruption of epithelial barrier integrity	100-300 μM	Human epithelial cell line Caco-2	Activation of p38 and p42/44 kinases	21
	Stimulation of the pro-inflammatory mediators COX-2, KC, IL-6, and IL-1α	10-200 μM	Murine embryonic fibroblast cell line NIH3T3 and human alveolar epithelial cell line A549	Inhibition of PPARγ	6
	Repression of NF-κB–responsive genes	5-50 μM	Bone marrow-derived macrophages, murine embryonic fibroblasts, alveolar macrophages, normal human bronchial epithelial cells	Modulation of NF-κB regulation	34
	Apoptosis	1-100 μM	Human Jurkat T lymphocytes, CCRF-CEM T lymphocytes, THP-1 human monocytes	Activation of the intrinsic mitochondrial pathway	17
	Inhibition of sperm viability and function	1-100 μM	Human spermatozoa	None	20
	Inhibition of proliferation, apoptosis, histamine release, degranulation and modulation of IL-6	6 -100 μM	Murine mast cell lines P815 and human mast cell line HMC-1	Intracellular Ca^{2+} release	18
	Regulation of accumulation of multiple proteins	10 nM – 2 μM	*Medicago truncatula* roots	None	43
	Alteration of mimic compound exudation	2 nM	*Medicago truncatula* roots	None	43
	Induction of auxin response gene *GH3* Cell specific induction of chalcone synthase	50 μM	White clover *(Trifolium repens)* roots	None	43
	Reduced zoospore swimming speed	25 and 125 μM	*Enteromorpha (Ulva)* zoospores	None	42
 $3OC_{16:1}$-HSL	Regulation of accumulation of multiple proteins	1 nM – 2 μM	*Medicago truncatula* roots	None	43

in this area has focused on elucidating the effects of AHL autoinducers produced by the opportunistic pathogen *Pseudomonas aeruginosa* on mammalian cells. The main reasons for the intense focus on this particular species are because its QS systems are exceptionally well-characterized and have been shown to control pathogenesis in many model systems.[9]

P. aeruginosa coordinates the production of many potent virulence factors by quorum sensing; thus knocking out these systems deals a crippling blow to its virulence.[9] This has made *P. aeruginosa* an extremely attractive target for developing quorum sensing inhibitors as potential therapeutics.[10,11] However, this intense study also led to a focus on the AHL autoinducers themselves as potential modulators of host responses.[7] To date, these studies have primarily focused on the *N*-(3-oxododecanoyl)-L-homoserine lactone ($3OC_{12}$-HSL) autoinducer made by *P. aeruginosa* (Table 1) and can be divided into two general categories: loss of cell integrity and immune modulation.

2.1. Mechanisms of AHL-Induced Cell Death

Programmed cell death, or apoptosis, is a protective cellular mechanism that prevents the damage that can be incurred when neighboring cells undergo unregulated cell lysis, such as in necrotic cell death. Many bacteria, including *P. aeruginosa,* produce virulence determinants that modulate host cell apoptotic cascades and induce cell death,[12,13] leading some to speculate that this ability in itself may be a potentially powerful host evasion strategy.[14] However, while QSS-triggered apoptosis potentially allows the pathogen to avoid host defense, it could also represent the ability of the host to recognize the QSS of potential pathogens to initiate its own defenses. The issue of "who benefits", the microbe or the host, is likely to depend on the nature of the relationship (pathogenic, symbiotic or commensal) and the predisposition of the host (healthy or diseased).

It has been well documented that the *P. aeruginosa* AHL $3OC_{12}$-HSL induces apoptosis in several different types of mammalian cells including macrophages and neutrophils,[14] breast cancer cells,[15] fibroblasts and endothelial cells,[16] lymphocytes and monocytes,[17] and mast cells[18] *in vitro*. For the most part, apoptosis was detected when these cells were exposed to relatively high concentrations of $3OC_{12}$-HSL (Table 1) but not when exposed similar levels of other AHLs including C_4-HSL, C_5-HSL, C_6-HSL, C_8-HSL, C_{10}-HSL, C_{12}-HSL, $3OC_6$-HSL, $3OC_7$-HSL, $3OC_8$-HSL, or the D-$3OC_{12}$-HSL enantiomer[14,16,17] indicating that this response is AHL-structure-specific. Other types of cells appeared to be resistant to the apoptotic effects of $3OC_{12}$-HSL. Specifically, several lines of epithelial cells, including CCL-185, A549, and HEp-2 cells, did not undergo apoptosis in response to $3OC_{12}$-HSL exposure [14,16] It is not yet clear why some types of cells are sensitive to $3OC_{12}$-HSL while others are not, but these data could help point to the identity of the signaling pathways and host-cell receptors involved. Thus far, even among $3OC_{12}$-HSL-sensitive cell lines there appear to be different apoptotic mechanisms involved, which are discussed below.

2.1.1. $3OC_{12}$-HSL and the Mitochondrial Pathway

One mechanism by which cells initiate apoptosis is by the intrinsic or mitochondrial pathway, so-called because it is initiated by internal signals within the cell, which result in damage to the mitochondria. Intracellular damage inhibits the protective effect of Bcl-2 (B-cell lymphoma 2) on mitochondria, resulting in membrane damage and the release

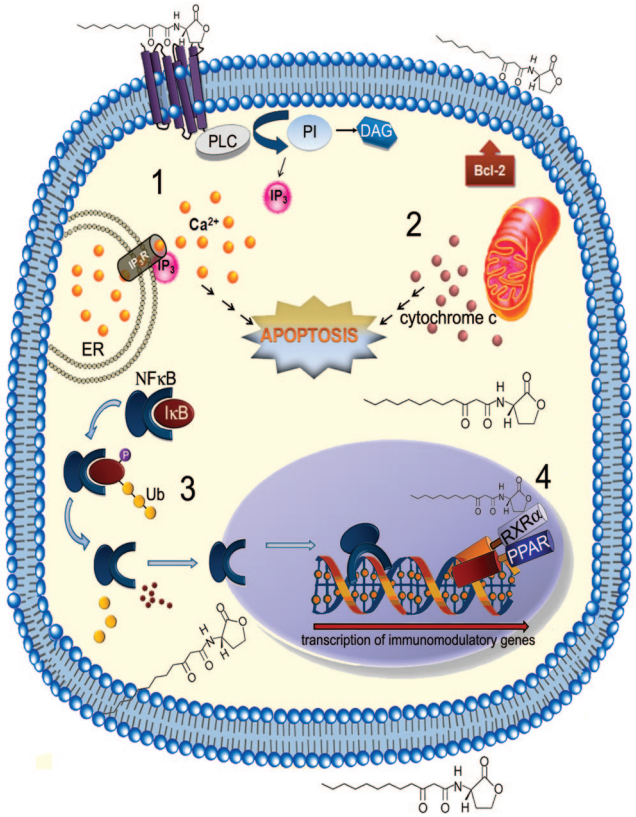


Figure 1. Mechanisms mediating AHL effects on mammalian cells: (1) AHL-induced increases in cytosolic calcium have been linked to apoptosis in fibroblasts and mast cells and are postulated to be the downstream effect of $3OC_{12}$-HSL binding at or near the cell membrane. (2) Intracellular damage inhibits the protective effect of Bcl-2 on mitochondria, resulting in membrane damage, the increase of cytochrome *c*, and apoptosis in T cells and macrophages exposed to $3OC_{12}$-HSL. (3) NF-κB has been demonstrated to be a key transcriptional factor, indirectly mediating many of the immunomodulatory effects of $3OC_{12}$-HSL. (4) The nuclear hormone receptor PPARγ was the first eukaryotic receptor shown to directly interact with an AHL. Acting as a heterodimer with RXRα, PPARγ binds to DNA at specific PPAR response elements within the promoters of target genes, activating their transcription.

of cytochrome *c* (Figure 1). This in turn sets into motion a cascade of signaling events involving caspases-9, 3, and 7 that increases cellular proteolytic activity and eventually results in the digestion of structural proteins in the cytoplasm, degradation of DNA, and phagocytosis. Signals external to the cell can also set apoptosis into motion, via the extrinsic or death receptor pathway. This pathway is classically initiated by the binding of Fas-ligand or tumor necrosis factor to their cognate integral membrane protein receptors, which results in the activation of caspase 8 and also eventually leads to phagocytosis of the apoptotic cell.

In a seminal paper documenting the apoptotic effects of AHLs, macrophages and neutrophils that were exposed to $3OC_{12}$-HSL demonstrated an increase in the activation of both caspases 3 and 8,[14] leading the authors to speculate that the extrinsic pathway was involved. However, later data obtained by exposing T lymphocytes to $3OC_{12}$-HSL clearly demonstrated that apoptosis was rapidly induced as early as one hour after $3OC_{12}$-HSL treatment and caused a breakdown in the mitochondrial transmembrane potential.[17] These apoptotic effects were specifically inhibited by overexpressing Bcl-2 in T lymphocytes.[17] In addition, electron microscopy of $3OC_{12}$-HSL-treated macrophages revealed dramatic mitochondrial swelling and distension of the endoplasmic reticulum.[19] These cellular alterations were only seen when cells were exposed to the naturally occurring $3OC_{12}$-L-HSL stereoisomer, and this provided further support for the involvement of the mitochondrial pathway in AHL-induced apoptosis.

2.1.2. Increases in Intracellular Calcium Induced by $3OC_{12}$-HSL

While mitochondrial damage may be a key step in the induction of apoptosis by $3OC_{12}$-HSL in lymphocytes, other groups have uncovered a role for intracellular calcium mobilization as an early signal transduction event that precedes apoptosis. Shiner et al. demonstrated that treatment of mouse fibroblasts with $3OC_{12}$-HSL resulted in a rapid increase in the concentration of cytosolic calcium, which correlated with apoptosis.[16] Using specific pharmacological inhibitors of key components in calcium signaling pathways, they demonstrated that calcium was mobilized from intracellular stores in the endoplasmic reticulum upon $3OC_{12}$-HSL treatment and could be blocked by inhibiting phospholipase C, suggesting that release involved inositol triphosphate (IP_3) receptors in the endoplasmic reticulum (ER). This led the authors to speculate that the most likely candidate receptors for mediating this response were located at or near the cell membrane. Interestingly however, while blocking the release of intracellular calcium protected cells from the apoptotic effects of $3OC_{12}$-HSL, it did not inhibit its immunomodulatory effects,[16] suggesting the existence of at least two host-cell receptors that mediate $3OC_{12}$-HSL effects. It was later shown that increases in intracellular calcium accompanied apoptosis in $3OC_{12}$-HSL-treated mast cells as well,[18] although a direct link between the two was not determined.

Human sperm cells were also susceptible to AHL-induced apoptosis, when exposed to $3OC_{12}$-HSL.[20] Cell death was preceded by the loss of the acrosome structure on the sperm head, which is essential for fertilization. Acrosome loss occurred when cells were exposed to relatively low concentrations of $3OC_{12}$-HSL ($1-5$ μM) and were dependent on the mobilization of intracellular calcium. Thus, when cells were co-incubated with $3OC_{12}$-HSL and the calcium chelator EDTA, the effect of $3OC_{12}$-HSL on sperm acrosomes was abolished.[20] Taken together, these data may indicate a conserved mechanism by which $3OC_{12}$-HSL affects a subset of host cells and could point to a specific class of host receptors mediating these apoptotic effects.

2.1.3. Loss of Cell Integrity

In addition to their apoptotic effects, AHLs also have more subtle documented effects on the structural integrity of mammalian cells, which could contribute to the virulence of *P. aeruginosa*. For example, Vikström et al. observed that $3OC_{12}$-HSL disrupted the barrier integrity of human epithelial Caco-2 cells.[21] Gut epithelial cells exposed to $3OC_{12}$-HSL demonstrated significant reorganization of their actin cytoskeletons, lowered transepithelial electrical resistance, and reduced expression of key tight-junction proteins in comparison to vehicle-treated cells.[21] These effects were later demonstrated to be caused by $3OC_{12}$-HSL-induced alterations in the phosphorylation states of several tight-junction proteins[22] and could help explain *P. aeruginosa* invasion and translocation from the gut.

Other studies have reported alterations in the expression of different host receptors upon exposure to AHLs. Very

low concentrations of $3OC_{12}$-HSL (0.3−0.5 pM) inhibited the expression of two specific nucleotide receptors, P2Y2 and P2Y4, in airway epithelial cells.[23] Interestingly, this inhibition was only seen in epithelial cells lacking a functional cystic fibrosis transmembrane conductance receptor (CFTR) and was reversed when the CFTR protein was expressed by an adenovirus vector or cells were treated with specific anti-inflammatory drugs. The authors speculated that the expression of P2 receptors might be continuously repressed in the airways of CF patients that are persistently colonized by *P. aeruginosa*, which could help explain the deficiencies in mucociliary clearance. Later data suggested that *P. aeruginosa* AHLs actually increase the production of mucus in the lung. Imamura et al. reported that mucin-5AC (MUC5AC) mRNA and protein levels were increased in NCI-H292 human lung epithelial cells exposed to $3OC_{12}$-HSL.[24] This effect was dose responsive and could be inhibited by pretreating cells with azithromycin, which is known to reduce mucus hypersecretion.

Taken together, these data indicate that the *P. aeruginosa* AHL $3OC_{12}$-HSL not only can induce cells to undergo apoptosis by at least two different mechanisms but also can affect the host cell transcriptome, altering the expression of key receptors and structural proteins. While global changes in mammalian transcriptomes following AHL exposure remain to be determined, even subtle alterations in gene expression could have profound implications in the host response to *P. aeruginosa* infection, especially if underlying disease is a factor. It is especially alarming to consider how exquisitely sensitive CFTR-deficient lung epithelial cells were to $3OC_{12}$-HSL at physiologically relevant concentrations[23] and tempting to speculate how other disease-altered cells may be similarly affected.

2.2. AHL-Mediated Immune Modulation

Studies on the modulation of the host's immune response by AHLs tend to fall into one of two categories, those that report a pro-inflammatory response and those that demonstrate a suppression of the immune response. These opposing effects seem largely to depend on the identity of the responding cell type and the concentration of AHL used. For example, several different investigators have demonstrated that the *P. aeruginosa* AHL $3OC_{12}$-HSL induces a potent pro-inflammatory response in fibroblasts, endothelial and epithelial cells, keratinocytes, T cells, and macrophages and neutrophils *in vitro*,[6,16,25−27] as well as the intact mouse dermis *in vivo*.[28] These pro-inflammatory responses include increases in the expression of several cytokines and chemokines, including interleukins 1, 6, and 8 (IL-1, -6, and -8), tumor necrosis factor alpha (TNFα), and interferon gamma (INFγ), as well as other immunomodulatory factors such as cyclooxgenase-2 (COX-2) and macrophage inhibitory protein (MIP).

Overall, these effects have been dose responsive and specific for $3OC_{12}$-HSL, with maximal effects detected at relatively high concentrations of AHL (Table 1). Thus, it has been speculated that the AHL-induced increases in inflammation could result in significant damage to host cells in their immediate vicinity, which in turn could enhance the rapid local and systemic spread of *Pseudomonas* seen in some types of acute infections.[9]

Conversely, it has also been postulated that *P. aeruginosa* utilizes its autoinducers not only to control its own gene transcription and virulence but also to suppress the host's inflammatory response, creating an environment that favors chronic persistence, rather than acute systemic spread. Numerous reports have documented the immunosuppressive effects of $3OC_{12}$-HSL on lymphocytes, which include inhibition of proliferation and cytokine production in both mitogen-stimulated and antigen-stimulated T lymphocytes.[29−32] Collectively, these effects have been detected in cells exposed to lower concentrations of $3OC_{12}$-HSL (<10 μM). Taken together with data demonstrating that $3OC_{12}$-HSL modulates antibody production by B lymphocytes and inhibits cytokine production in lipopolysaccharide (LPS)-stimulated macrophages,[29] these data support the concept that $3OC_{12}$-HSL functions to shift the host's immune system to an anti-inflammatory Th-2 response. This could help explain how *P. aeruginosa* causes decades-long colonization in the lungs of cystic fibrosis patients without invading or spreading systemically.

Importantly, there appear to be structural components of AHLs required for maximal immunomodulatory activity. A systematic analysis examining the immunosuppressive activities of a series of synthetic analogues of $3OC_{12}$-HSL revealed that optimal activity was achieved with AHLs containing an intact HSL ring, acyl side chains with 11−13 carbons, and either a 3-oxo or 3-hydroxyl modification.[32] In addition, many of these studies have compared the effects of $3OC_{12}$-HSL on host cells to the other major AHL made by *P. aeruginosa* (C_4-HSL) or the $3OC_{12}$-D-HSL enantiomer. In all cases, only $3OC_{12}$-L-HSL yielded a pro-inflammatory effect.[6,16,32]

2.2.1. The Involvement of MAP Kinases and NF-κB Signaling in AHL Immunomodulation

The nuclear transcription factor NF-κB is a multifunctional regulator of diverse processes including inflammation, apoptosis, and development.[33] It exists in a latent form in the cytoplasm of unstimulated cells, where it is sequestered by the IκB proteins. Stimulation by cytokines, LPSs, and other products of infection results in phosphorylation, ubiquitylation, and degradation of IκB, freeing NF-κB to translocate to the nucleus where it regulates target genes (Figure 1). The role of NF-κB in the perception of AHLs by mammalian cells has been investigated in several studies over the past decade. Initially, Smith et al. identified NF-κB and activating protein 2 (AP-2) as two transcriptional regulators that potentially mediated the inflammatory effects of $3OC_{12}$-HSL in lung fibroblasts[25] and provided data that $3OC_{12}$-HSL induced both IL-8 and COX-2 through NF-κB regulation.[25,26] This group also demonstrated that two members of the MAP kinase family of serine/threonine kinases, ERK-1 and ERK-2 (also called p42/p44), were activated by $3OC_{12}$-HSL in lung epithelial cells and that NF-κB activation was blocked by pharmacologic inhibition of MAP kinase activity. These data thus suggested that the ERKs were upstream mediators of $3OC_{12}$-HSL-dependent NF-κB activation in these cells,[25] and that the effects of $3OC_{12}$-HSL on this MAPK/NF-κB pathway, which led to the induction of the potent chemokine IL-8 and increased migration of neutrophils, possibly contributed to a destructive inflammation seen in the respiratory tract of *P. aeruginosa* infected patients.

More evidence for the role of MAP kinases in perception of AHLs by host cells came from Vikstrom et al. who demonstrated that $3OC_{12}$-HSL stimulated a second MAPK pathway in macrophages.[27] Upon application of $3OC_{12}$-HSL to human macrophages, the MAPK p38 signaling pathway

was activated, which corresponded with increases in their phagocytic activity. Furthermore, pretreatment with a selective inhibitor of p38 MAPK prevented $3OC_{12}$-HSL-induced phagocytic activation of macrophages. Kravchenko et al. also demonstrated that exposure of bone marrow-derived macrophages to $3OC_{12}$-HSL resulted in a dose- and time-dependent induction of MAPK p38 and eIF2α phosphorylation.[19] These effects were highly specific and did not occur when $3OC_{12}$-D-HSL was applied. Interestingly, neither group detected ERK-1 and ERK-2 activation in $3OC_{12}$-HSL-stimulated macrophages, and the MAPK p38 signaling pathway was not activated in $3OC_{12}$-HSL-stimulated lung epithelial cells.[19,25,27] These data further illustrate that the effects of AHLs differ greatly depending on cell type.

The specific role of NF-κB in $3OC_{12}$-HSL immunomodulation was further investigated in a comprehensive study that examined the ability of $3OC_{12}$-HSL to affect signaling cascades in LPS-stimulated macrophages.[34] Initial experiments examining the responsiveness of macrophages exposed to *P. aeruginosa*, *Salmonella enterica* sv Typhimurium, or *Staphylococcus aureus* revealed alterations in IκB processing only in *P. aeruginosa*-exposed cells, and these alterations were not seen in cells exposed to a *P. aeruginosa lasI* mutant. Further experiments demonstrated that $3OC_{12}$-HSL inhibited LPS-mediated phosphorylation of IκB, which consequently inhibited NF-κB activity. However, $3OC_{12}$-HSL alone did not affect NF-κB signaling. These data suggested that $3OC_{12}$-HSL could have a role in dampening the inflammatory response of macrophages to bacterial products such as LPS and therefore contribute to the persistence of *P. aeruginosa* in chronic infections.

2.2.2. Host-Cell Receptors Mediating AHL Responses

The exquisite specificity of AHL action on mammalian cells implies the existence of specific receptor proteins that mediate their effects on mammalian gene expression. Considering that many microbial products are recognized by specific pattern recognition receptors (PRRs), which results in the activation of the transcription factor NF-κB and MAPK p38, Kravchenko et al. hypothesized that PRRs represented ideal candidates for mediating the immune effects of AHLs.[19] PRRs are membrane-bound or cytosolic receptors that recognize highly conserved pathogen-associated molecule patterns and trigger immune responses accordingly, and members of this group include the Toll-like receptors (TLRs).[19] However, a thorough survey of several PRR-dependent signaling proteins, using genetically null mice, revealed that the canonical Toll-like receptor pathways were not involved in AHL signaling.[19]

Although TLRs did not appear to mediate the effects of $3OC_{12}$-HSL in mammalian cells, another class of host-cell receptor may directly interact with bacterial AHLs. Jahoor et al. hypothesized that the peroxisome proliferator activated receptors (PPAR) could mediate the inflammatory effects of $3OC_{12}$-HSL.[6] PPARs are members of the mammalian nuclear hormone receptor family. There are three isoforms (PPARα, -β/δ, and -γ) each of which are ligand-activated transcriptional regulators that bind to DNA as obligate heterodimers with the retinoid X receptor (RXR) at specific PPAR response elements within the promoters of target genes. PPARs are widely expressed in human tissues, have broad roles in lipid metabolism, differentiation, and inflammation, and bind to a wide range of lipid-based endogenous and exogenous ligands.[6] Jahoor et al. investigated the effects of *P. aeruginosa* AHLs on the activities of the PPARβ/δ and -γ and demonstrated that $3OC_{12}$-HSL (but not C_4-HSL) significantly inhibited the transcriptional and DNA-binding activities of PPARγ *in vitro*. In addition, they were able to inhibit $3OC_{12}$-HSL-induced up-regulation of several proinflammatory cytokines in lung epithelial cells by adding rosiglitazone, a potent PPARγ agonist.[6] These data suggested that $3OC_{12}$-HSL and rosiglitazone compete for PPARγ binding. However, while rosiglitazone activates PPARγ upon binding, $3OC_{12}$-HSL acted as an antagonist.

These observations were recently supported when Cooley et al. showed that $3OC_{12}$-HSL effectively binds to the PPARγ ligand binding domain and interferes with the binding of rosiglitazone at concentrations as low as 1 nM.[35] Thus, PPARγ is the first documented eukaryotic receptor to directly interact with bacterial AHLs. Although these interactions have not yet been shown to occur *in vivo*, they may represent an important bacterial/host interactive mechanism, by which *P. aeruginosa* exploits a host receptor, using $3OC_{12}$-HSL to interfere with a normal cellular signaling. It is also interesting to speculate what potential effects AHLs may have on other members of the nuclear hormone family.

3. Recognition and Responses of Plants to AHL Signals

Plants are constantly exposed to bacteria, especially in the rhizosphere, the area immediately surrounding the roots, where plant exudates provide a food source for bacteria and where bacteria colonize the root surface. In addition, the leaf surface, the phyllosphere, is inhabited by various bacterial species, whereas bacterial endophytes inhabit the internal tissues of plants. For instance, nitrogen-fixing bacteria inhabit plant tissues either extracellularly, for example, in the apoplast of sugar cane, or intracellularly, for example, inside cells of nodules formed by rhizobia on legume roots. Communication of plant-associated bacteria by AHLs is crucial for interaction of plant pathogens and symbionts with hosts.[36,37] Common traits regulated in plant-associated bacteria include synthesis of virulence factors, degradative enzymes, and exopolysaccharides, regulation of nitrogen fixation genes, biofilm formation, and plasmid transfer.[37−39] A survey of bacterial species in soil found that quorum sensing is more common in rhizosphere bacteria than in those inhabiting the bulk soil.[40] Therefore, it is not surprising that recent studies have shown that plants can "eavesdrop" on and interfere with bacterial quorum sensing (Table 1, Figure 2).

The first evidence that plants can utilize the information of AHLs came from the green seaweed *Enteromorpha (Ulva)*, which forms zoospores that settle on bacterial biofilms. Joint et al. (2002) showed that the zoospores can detect AHLs from biofilm forming bacteria and that this enables them to settle on appropriate biofilms in marine environments.[41] The authors showed that zoospores only settled on biofilms of bacteria synthesizing appropriate AHLs and also used synthetic AHLs to stimulate zoospore attachment. Subsequently, it was shown that the AHLs $3OC_{12}$-HSL and $3OC_{10}$-HSL promote zoospore settlement by reducing the swimming speed of the zoospores around the AHL source, whereas the orientation of zoospores toward or away from an AHL source was not affected.[42] The molecular mechanism of detection and response to AHLs in the algal zoospores remains unknown.

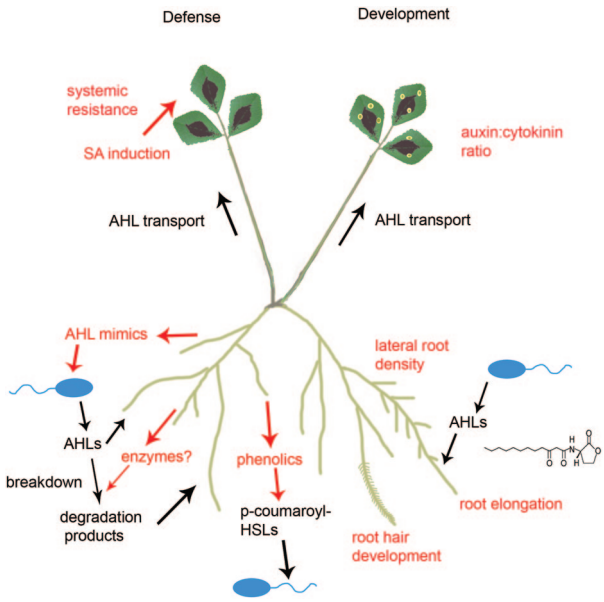


Figure 2. Overview of defense and developmental responses of plants to AHLs. AHL perception, shown here in the root, leads to the production of AHL mimics exuded back into the rhizosphere. In addition, induction of the phenylpropanoid pathway might lead to exudation of more phenolic metabolites, of which *p*-coumaric acid can be integrated into bacterial signals to form *p*-coumaryl-homoserine lactones (HSLs). In addition, the plant is likely to affect AHL stability by exuding degradative enzymes. AHLs can induce salicylic acid (SA) in roots and shoots, which is suggested to mediate systemic resistance to pathogens (indicated by lack of necrosis symptoms on left leaf). Certain AHLs also affect root development by enhancing root elongation, root hair formation, and lateral root density. Some developmental effects are due to changes in the concentrations and ratios of the plant hormones auxin and cytokinin in shoot and root. AHL responses can be local and systemic, and certain AHLs have been shown to move from roots to shoots.

The legume *Medicago truncatula* responds to nanomolar concentrations of synthetic and purified AHLs from its symbiont *Sinorhizobium meliloti* ($3OC_{16:1}$-HSL) and from *Pseudomonas aeruginosa* ($3OC_{12}$-HSL) with specific and extensive changes in root protein expression.[43] A proteomic study revealed that AHL-dependent plant proteins included a number of potential defense-related proteins, metabolic enzymes, and proteins involved in the recognition of plant hormones auxin and cytokinin. In addition, the expression of reporter constructs for chalcone synthase, the first enzyme of the flavonoid biosynthetic pathway, and an auxin response gene were induced in specific cell types in white clover (*Trifolium repens* L.) roots locally treated with AHLs.[43] For example, a reporter in the chalcone synthase gene, *CHS3*, was induced in inner cortical cells underlying the site of treatment but not in epidermal or outer cortex cells. This suggests that responses to AHLs are cell-type specific and that either the AHLs or secondary signals triggered by their perception can travel inside the root.

It was subsequently shown that treatment of roots with AHL-producing bacteria (*Serratia marcescens*) but not their AHL-deficient mutants increased systemic resistance against the fungal leaf pathogen *Alternaria alternata* in tomato shoots.[44] In addition, treatment of roots with synthetic AHLs (C_6-HSL) enhanced the expression of typical defense-related genes in untreated leaves of tomato, these included pathogenesis-related and ethylene-inducible proteins. It is possible that the systemic effects of AHLs are mediated by the induction of salicylic acid, which was increased in roots and shoots of plants treated with AHL-producing bacteria and synthetic AHLs.[44]

A transcriptomic approach in *Arabidopsis* showed gene expression changes for several hundred genes in shoots and roots in response to 10 μM concentrations of C_6-HSL.[45] This study showed evidence for the induction of auxin response genes and increased auxin levels in roots and shoots of C_6-HSL-treated plants, whereas cytokinin responses and concentrations were reduced. In addition, other genes involved in plant development were altered, for example, arabinogalactan proteins, hydroxyproline-rich glycoproteins, expansins and extensins, and genes involved in responses to plant hormones gibberellin and ethylene. Treatment with C_6-HSL and C_4-HSL increased root growth in *Arabidopsis*, whereas longer chain AHLs reduced root growth, while shoot growth appeared not to be affected.[45] In contrast to the aforementioned induction of systemic resistance by AHLs in tomato, AHL-treated *Arabidopsis* showed no enhanced resistance toward *Pseudomonas syringae* and only weak induction of typical defense related genes.

A different study with *Arabidopsis* demonstrated that growth of plants on AHL containing medium inhibited root growth, depending on the acyl side chain length of the AHL.[46] In addition, some AHLs, in particular, C_{10}-HSL, increased lateral root density and accelerated emergence in *Arabidopsis* and caused changes in root hair development, although at fairly high concentrations (48−192 μM). The lateral root and root hair induction close to the root tip could indicate that the AHLs have caused a termination of the apical root meristem, and this was supported by reduction in the expression of cell cycle markers.[46] These responses of C_{10}-HSL were deemed unlikely to be mediated by auxin signaling, because C_{10}-HSL did not increase auxin responses measured with the auxin response gene *DR5:GUS*, and the auxin response mutants *aux1*−*7*, *axr2*, and *doc1* showed similar root length and lateral root responses to C_{10}-HSL as the wild type.

Whether the developmental responses to AHLs are relevant in nature in response to local AHL concentrations remains to be shown. Studies in which bacterial AHL synthesis genes were transferred to plants as a strategy to interfere with bacterial quorum sensing showed that plants can accumulate AHLs in their tissues. For example, low micromolar concentrations of $3OC_6$-HSL were produced in tobacco transformed with the *yenI* gene of *Yersinia enterocolitica*.[47] No gross developmental alterations in the *yenI* transgenics were reported,[47−49] although it would be interesting to specifically test for developmental changes in these plants.

Overall, the studies show that plant responses to AHLs depend on AHL structures and concentrations and on the plant species, that responses vary between root and shoot, that responses can be systemic as well as cell type specific, and that responses involve defense-related as well as developmental changes. The response of plants to AHLs also depends on movement and transport in the plant and AHL distribution and concentration on plant surfaces. AHLs appear to enter and move systemically in plant tissues. Mass spectrometric detection of C_6-HSL and C_{10}-HSL in *Arabidopsis* showed that C_6-HSL moved into roots and shoots and accumulated in shoots within four days after treatment of roots with AHLs, whereas the more hydrophobic C_{10}-HSL remained mainly in the root.[45] Likewise, C_6-HSL was found to accumulate in shoot of root-treated barley (*Hordeum*

vulgare L.) and yam bean (*Pachyrhizus erosus* L. (Urban)) while longer chain AHLs were less abundant in shoots.[50] Roots of barley and yam bean also discriminated between chiral forms of AHLs, preferring the L-isomers over D-isomers.[50] While this specificity argues for the existence of an AHL receptor in plants,[51] there is no evidence that plants encode receptor proteins similar to those of bacterial AHL receptors. Likewise, no other type of plant AHL receptor has been identified thus far.

The ability of plants to detect AHLs from the rhizosphere could be advantageous to plants in order to intercept pathogens. It is thought that quorum sensing regulated expression of elicitors or virulence factors, for example, presents a strategy for bacteria to avoid early detection by the plant host because elicitors would only be produced after high densities of bacteria are present. However, if plants can detect low concentrations of AHLs they might be able to respond before pathogen numbers build up. This concept would have to be tested in the future with plant mutants unable to respond to AHLs. Furthermore, future studies will need to investigate plant responses to QSS other than AHLs, which have so far not been investigated.

4. AHL Signal Mimics from Plants and Their Role in Modulating Plant−Bacterial Interactions

Since bacterial pathogens and symbionts rely on QS regulation to optimize their infection of host organisms, it is not surprising that eukaryotic hosts have evolved mechanisms to perceive bacterial QS signals, respond to them, and also manipulate bacterial cell-to-cell signaling. Most of the QS-active compounds produced by plants, algae, and fungi disrupt bacterial QS regulatory cascades and inhibit behaviors controlled by QS. For example, halogenated furanones produced by a red alga *Delisea pulchra* inhibit expression of QS-controlled virulence genes and corresponding behaviors in plant and animal pathogens.[52] Vesicle-mediated release of halogenated furanones to the surfaces of algal thalli modulates the composition of the associated microbiota and shifts it from the community that is dominated by Gram-negative bacteria (common in marine environments) to those dominated by Gram-positive bacteria.[53] The ability of halogenated furanones to affect the associated microbial communities was the first strong evidence that these QS-active signals produced by eukaryotes have coevolved functions in eukaryote−bacterial interactions.

Red algae are not unique in their ability to manipulate bacterial QS and affect QS-dependent behaviors in the associated microbiota. Seedlings of many plants, including garlic, pea, rice, tomato, soybean, and *Medicago* spp. secrete compounds that can inhibit AHL receptor-dependent responses in QS reporter bacteria.[43,54−58] The plant compounds appear to be secreted at levels that would affect QS regulation in associated bacteria during natural encounters.[54,57] In contrast to the red algae, which only produce halogenated furanones that broadly inhibit QS in the associated bacteria, terrestrial plants produce a suite of compounds, some of which inhibit QS while others stimulate QS.

The first eukaryotic compound structurally unrelated to AHLs and capable of stimulating bacterial QS was identified as lumichrome, a derivative of the vitamin riboflavin[59] (Figure 3). Lumichrome and riboflavin likely directly interact with the AHL-binding pocket of LasR, the LuxR-type AHL receptor of *P. aeruginosa.* The AHL-binding pocket of LasR shares at least three conserved amino acid residues (Y56, W60, and D73) with LuxR of *Vibrio fischeri* and TraR of *Agrobacterium tumefaciens*.[60−62] Y56, W60, and D73 are involved in hydrogen bonding to the 1-carbonyl, the ring carbonyl, and the NH group (respectively) of $3OC_{12}$-HSL.[63]

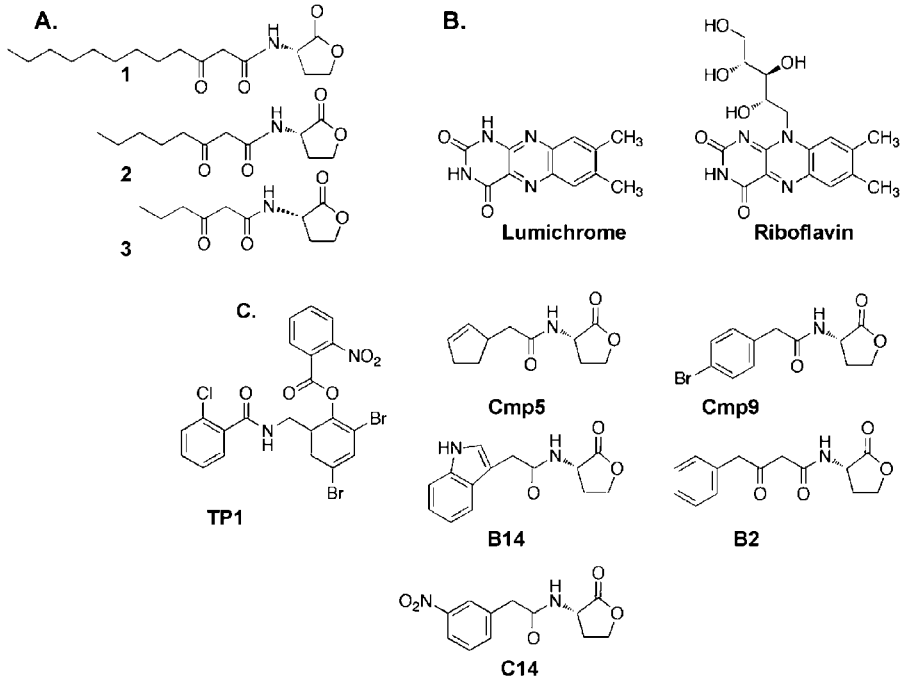


Figure 3. Bacterial AHL signals and their naturally occurring and synthetic mimics. (A) AHL signals used by proteobacteria: (1) $3OC_{12}$-HSL, a ligand for LasR AHL receptor of *P. aeruginosa*; (2) $3OC_8$-HSL, a ligand for TraR AHL receptor of *A. tumefaciens*; (3) $3OC_6$-HSL is a common AHL produced by *Vibrio fischeri*, *Pectobacteria* spp., and *Yersinia* spp. In *V. fischeri*, $3OC_6$-HSL is recognized by LuxR AHL receptor. (B) Stimulatory AHL mimics lumichrome and riboflavin are produced by bacteria, single-cell algae, and plants. Lumichrome and riboflavin appear to interact with the AHL-binding domain of LasR. (C) Synthetic QS agonists and antagonists identified in refs 62, 64, 66, and 138.

Consistent with the proposed roles for these residues in the interactions of LasR with $3OC_{12}$-HSL as well as lumichrome and riboflavin, LasR proteins with missense mutations D73N and W60F were not responsive to these ligands.[59] Interestingly, the Y56F mutation in LasR had relatively little effect on responses of the LasR-dependent *lasI* reporter to $3OC_{12}$-HSL, but it substantially reduced responses of another LasR-dependent reporter (*rsaL*) to the cognate AHL. The *lasI* reporter in the LasR-Y56F background responded much more strongly to the added riboflavin and lumichrome than the reporter in the wild-type LasR background.[59] It appears, therefore, that the Y56F missense mutation may selectively enhance lumichrome or riboflavin binding or activation of the receptor for *lasI* expression without greatly affecting induction of *lasI* by $3OC_{12}$-HSL. In addition to being involved in interaction of LasR with the cognate AHL, lumichrome, and riboflavin, Y56, W60, and D73 and S129 are also required for binding of the synthetic compounds B14, cmp5, and cmp9 within the AHL-binding pocket of LasR (Figure 3).[62,64] However, unlike $3OC_{12}$-HSL or the agonist AHL mimics (lumichrome and riboflavin), B14, cmp5, and cmp9 were broadly inhibitory to the LasR- and TraR-based reporters.[62,64]

Even though AHL-binding sites of the LuxR-type AHL receptors share some similarity, not all of them are susceptible to manipulation by plant AHL signal mimics *in situ.* For example, root exudates of aseptically germinated tomato seedlings excreted compounds that affected the SwrR-based reporter of *Serratia marcescens* and had modest effect on the LasR-based reporter, while the CepR reporter of *P. putida* was not affected in the tomato rhizosphere.[54,65] The observation that AHL receptors respond differently to plant AHL mimics is consistent with the results of experiments that demonstrated that same synthetic AHL analogues could inhibit, promote, or have no effect on different AHL receptors.[64] For example, compound B2 (Figure 3) activated the *P. aeruginosa* LasR-based reporter and had a modest inhibitory effect on both the *V. fischeri* LuxR-based and the *A. tumefaciens* TraR reporter; compound C14 strongly activated the LuxR reporter, inhibited LasR, and had a modest inhibitory effect on the TraR reporter.[64] TP-1 (and its derivatives TP-2, TP-3, TP-4, and TP-5, Figure 3) stimulated the LasR reporter but had no effect on the three other *P. aeruginosa* AHL receptors.[66] The ecological consequences of the differential sensitivity of AHL receptors to manipulation by AHL mimics are not yet certain. An answer to this question will be possible once plant mutants defective in AHL mimic synthesis are identified or constructed.

In addition to lumichrome and riboflavin, plants and terrestrial algae produce a number of yet unidentified QS-active substances capable of stimulating bacterial QS. *M. truncatula*, for example, produces more than a dozen chromatographically separable compounds that stimulate or inhibit QS responses in the LasR- or LuxR-based reporters.[55] The production of a diversity of AHL mimics by plants may not be surprising, considering that the red alga *D. pulchra* has been reported to synthesize about 30 different halogenated furanones at various concentrations.[67] It is unlikely, however, that all of the *M. truncatula* mimics likewise belong to a single class of compounds: some (but not all) AHL mimics excreted by this plant likely contain a lactone ring, because they were sensitive to degradation by an AHL lactonase, AiiA.[68]

The dynamics of AHL synthesis and secretion is another important yet poorly understood phenomenon relevant to the question of host manipulation of bacterial QS *in situ*. It appears that the synthesis and secretion of AHL mimics depend on the developmental stage of the host and the presence of AHL-producing bacteria. Exposure of *M. truncatula* seedlings to nanomolar levels of AHLs led to the appearance of additional chromatographically separable peaks of QS activity in their exudates.[43] It is possible that some of these new peaks represent QS signal mimics produced in response to bacterial AHLs while others may represent bacterial AHLs modified by the plant AHL-degrading enzymes.[69] Alternatively, the appearance of new activities may be due to the new AHL mimics synthesized by the plant in response to bacterial AHLs. The synthesis or secretion of AHL mimics by plants appears to be developmentally regulated. Seeds and seedlings of *M. truncatula* secreted different mimics or substantially different amounts of the same mimics at different stages of development.[55] Many of the QS-active compounds in the exudates from seeds and young seedlings subsequently became undetectable.[55] This suggests that the plant stops either making or secreting them and that the initially secreted mimics are subsequently degraded or inactivated. The induced synthesis or secretion of QS active compounds by plants at different developmental stages and in response to bacterial QS signals may prove to be an important aspect of many plant−bacterial interactions. The biological significance of these changes in secreted QS active compounds for interactions with naturally encountered bacteria remains to be investigated.

In addition to QS signal mimics, L-canavanine, an arginine analog exuded in large amounts by seeds of alfalfa (*Medicago sativa* L.), was shown to inhibit AHL signaling in the *Chromobacterium violaceum* reporter. L-Cananavine also inhibited QS-mediated production of exopolysaccharide (EPS-II) in *Sinorhizobium meliloti,* an alfalfa symbiont.[57] EPS-II is important for plant host infection and nitrogen fixation.[57] It is likely that L-canavanine does not directly compete with AHLs for their receptors; rather as an L-arginine analog, L-canavanine is incorporated into proteins (like AHL receptors), hindering their proper folding. This means that the effect of L-canavanine on AHL-regulated behaviors might be general and not specific. Another explanation for the effect of L-canavanine could be that some bacteria can break it down to homoserine, which could then be converted to an AHL mimic.[57] It is of note, however, that neither homoserine nor homoserine lactone are known to affect bacterial QS reporters.

Other plant secondary compounds with AHL inhibitory activities include salicylic acid (SA), *p*-coumaric acid, and the flavonoid catechin. SA, a plant metabolite that regulates plant defense responses, can cause breakdown of AHLs by stimulating expression of the AHL lactonase encoded by the *attKLM* operon in *Agrobacterium tumefaciens*.[70] *p*-Coumaric acid, a phenolic compound produced by plants as a lignin precursor and often exuded into the soil, inhibited activity of several QS biosensors in a concentration-dependent manner.[71] Interestingly, *p*-coumaric acid could contribute to quorum sensing in a more intriguing way by being integrated into a newly discovered quorum sensing signal, *p*-coumaroyl-homoserine lactone.[72] Therefore *p*-coumaroyl-HSL could have dual functions, to control density-dependent behaviors in bacteria as well as sense the proximity of a host plant.[72] Complicating this view is that *p*-coumaric acid can also be

produced by bacteria as a breakdown product of flavonoids, which themselves are common in root exudates.[73] The flavonoid catechin has recently been identified as a potential quorum sensing mimic molecule isolated from bark of *Combretum albiflorum*, a medicinal plant with antimicrobial properties.[74] Catechin inhibited an RhlR-based biosensor and reduced expression of RhlR-regulated genes, suggesting that catechin interferes with perception of C_4-HSL by RhlR. However, the concentrations of catechin needed for these assays were high and ranged between 0.125 and 4 mM. Therefore, it will be important to determine in the future whether these catechin concentrations are reached on plant surfaces colonized by bacteria.

5. AHL Degradation and Its Consequences to Structuring of Host-Associated Microbial Communities

5.1. AHL Degradation in the Rhizosphere

In understanding ecological consequences of recognition of bacterial AHL signals by eukaryotes, it is important to address the question of whether AHLs are actually produced during normal interactions between hosts and their associated microbiota. The answer to this question depends on the system examined. In plant−bacterial systems, for example, AHLs are produced by plant pathogens, symbionts, and commensals, where AHL-mediated QS controls behaviors that are central to the outcome of the interactions.[38,75,76] Fluorescent and recombinase-based *in vivo* reporters demonstrate that AHLs are produced and detected by bacteria in the plant root zone (rhizosphere)[65,77] and on leaf surfaces.[78] Therefore, synthesis of AHLs occurs under field conditions in plant-associated bacteria.

In the rhizosphere, AHL concentration is affected by adsorption to soil particles, opening of the lactone ring via photocatalyzed oxidation, or alkaline pH of the surrounding soil, as well as breakdown by plant and bacterial enzymes. Calculations of the half-life of AHLs at different temperatures and pH values ranged from hours at alkaline pH and warm (>20 °C) temperatures to many days under cold (<4 °C) and acidic conditions.[69] Under very alkaline conditions, stability of AHLs could be in the range of minutes due to lactolysis,[79] but lactolysis is reversible under acidic conditions. In addition, the AHL $3OC_{12}$-HSL can undergo Claisen-like condensations in aqueous environments, leading to the formation of the antimicrobial compound 3-hydroxydecylidene 5-(2-hydroxyethyl)pyrrolidine 2,4-dione, which is active against some Gram-positive bacteria,[80] suggesting that 3O-AHLs could be converted from a signal to a "weapon" against other microbes. Tetramic acids also have high affinity for iron and could be used to sequester iron from the rhizosphere. Ecological implications of this observation are considered below.

As a potential strategy to interfere with quorum sensing, plants can break down AHLs enzymatically. A study with different plants showed that AHLs are less stable around roots than in bulk soil and that AHL destruction is plant-specific, AHL-specific, and temperature-sensitive, suggesting enzymatic degradation of AHLs by plant roots or root exudates.[69] An *Arabidopsis* fatty acid amide hydrolase could be degrading AHLs, because overexpressing mutants were more resistant to AHLs whereas a fatty acid amide hydrolase mutant was more susceptible to developmental changes by AHLs.[46]

5.2. Animal Paraoxonases Hydrolyze AHLs and Contribute to Defense against Bacterial Pathogens

Degradation of bacterial AHLs is one of the mechanisms by which animals deal with bacterial QS. The ability to inactivate bacterial AHLs by hydrolyzing the lactone ring was found in mammalian sera but not in chicken or fish.[81,82] The degradation of AHLs appears to be specific. In human epithelial cell cultures, $3OC_{12}$-HSL and C_6-HSL were degraded essentially completely, while C_4-HSL and $3OC_6$-HSL remained largely unaffected after exposure to several kinds of mammalian cell lines.[83] Subsequent experiments demonstrated that airway epithelial cells produce paraoxonases PON1, PON2, and PON3, enzymes responsible for lactonolysis of $3OC_{12}$-HSL.[82,84]

Mammalian paraoxonases (PONs) are a family of calcium-dependent six-bladed β-propeller esterases, which consists of PON1, PON2, and PON3. They share 65% amino acid identity and are highly homologous (79−95%) to PONs from other mammals.[85] PONs have partially overlapping but also substrate-specific organophosphatase, arylesterase, and lactonase activities.[85] Even though weak AHL degrading activities are found in a variety of animal proteins (including human albumins, porcine carboxylesterases, and *N*-acylases), paraoxonases are the dominant enzymes responsible for the degradation of bacterial AHLs.[81,82,84,86]

It is not clear whether PONs have evolved to degrade bacterial AHLs or the ability to degrade AHLs results from the promiscuity of the enzymes. In considering this question, one needs to keep in mind that lactonase activities of PON1 and PON3 toward AHLs are ∼0.003−0.09 U/mg of the enzyme, while at least a dozen other lactones are degraded at least 1000 times more efficiently. However, human paraoxonases hydrolyze L- (and not D-) enantiomers of AHLs,[85] suggesting a specificity toward naturally occurring AHL isomers. PON2 was 10−100 times more efficient than PON1 or PON3 at hydrolyzing AHLs and did not efficiently use other lactones as substrates,[84,85,87] suggesting that PON2 may be the enzyme responsible for AHL degradation.

Consistent with the hypothesis that PON1 and PON3 are not critical to the degradation of AHLs, cell lysates prepared from tracheal epithelial cells of PON1 or PON3 knock out mice were indistinguishable from the wild-type cell lysates in the AHL-degrading activities.[84] However, overexpression of either PON1 or PON3 in CHO lines endowed the transfected cells with AHL degrading abilities.[82] PON2 knockout epithelial cell lysates were significantly impaired in their ability to degrade $3OC_{12}$-HSL, with 75% of unhydrolyzed $3OC_{12}$-HSL present at the end of a 60-min incubation.[87]

Because PONs have been shown to be effective at degrading AHLs produced by pathogens like *P. aeruginosa*, therapeutic applications of PONs have been explored. Expression of PON1 in fruit flies (which do not make own PONs) significantly reduced mortality of the animals infected with the wild-type *P. aeruginosa* and *Serratia marcescens*.[87] Expression of PON1 in fruit flies did not protect animals against *Staphylococcus aureus*, a Gram-positive pathogen that does not produce AHLs.[87]

Since a point mutation (S311C) in human PON2 occurs naturally, the role of this enzyme in the interactions of humans with bacteria can be investigated. Airway epithelial samples from donors carrying PON2-S311C were defective in degrading AHLs.[88] These observations indicate that even though PON1, PON2, or PON3 may not be solely responsible for AHL degradation, the three enzymes have the AHL lactonase activity, with PON2 likely playing the major role in AHL degradation.

5.3. AHL Turnover by Host-Associated Microbiota

Eukaryotic and prokaryotic microbes that are a part of normal microbiota associated with plants and some animals can break down AHLs and their degradation products. So far, there is no report of identification of AHL-degrading microbes directly isolated from the commensal microbiota associated with mammals. It is possible that the lack of AHL-degrading capabilities in the commensals of mammals is because various mammalian cells and tissues produce PON enzymes capable of degrading AHLs (as discussed above), while invertebrates and fish may have evolved to rely on their associated microbial communities for AHL degradation. Alternatively, mammals may rely significantly on the perception of AHLs to fine-tune responses to quorum sensing pathogens, and therefore AHL-degrading capabilities have been counterselected from the commensals.

The majority of the AHL-degrading microorganisms characterized to date have been isolated from the plant rhizosphere or soil. Many AHL-degrading bacteria also themselves use AHLs for their QS, and roles for these enzymes in QS signal turnover have been reviewed recently.[89] Therefore, here we focus mostly on the AHL-degrading bacteria that are not known to extensively rely on AHLs for their QS.

The plant rhizosphere is the environment where AHL-producing pathogens and commensals are most commonly found.[40] AHL-degrading strains are taxonomically diverse and include true fungi (ascomycetes and basidiomycetes), Firmicutes, and Gram-negative bacteria.[90−95] Under laboratory conditions, AHL-degrading bacteria protect their plant hosts from the pathogens that utilize AHLs for controlling virulence genes.[90,92−94,96,97] However, they also disrupt AHL-dependent synthesis of fungicides in biocontrol soil pseudomonads.[90,98] These reports indicate that AHL degradation by the rhizosphere bacteria affects diverse behaviors in host-associated microbiota and not all consequences of AHL degradation are beneficial to the plant host. These implications should be kept in mind as applications of AHL-degrading bacteria and transgenic plants are explored in agriculture.

AHL-degrading microbes have also been recovered from the microbial communities associated with fish and invertebrate animals. Polymicrobial consortia recovered from intestines of healthy white shrimp juveniles were isolated based on their ability to degrade AHLs within 24−48 h and multiply by 10^2 cfu/mL in AHL-containing medium.[99] Inoculation of a model rotifer *Brachionus plicatilis* with the AHL-degrading consortium afforded partial protection of the rotifer from an aquatic pathogen *Vibrio harveyi*, which uses AHL-mediated quorum sensing as one of the sensory inputs for the regulation of virulence genes.[99] An AHL-degrading *Shewanella* spp. has been isolated by enrichment from fish intestines and was shown to inhibit QS-regulated behaviors in a fish pathogen, *Vibrio anguillarum*.[100] Applications of AHL-degrading marine bacteria are being explored for biological control of aquaculture pathogens.[101]

Some bacteria are capable of completely degrading AHLs through a combined action of several enzymes.[92,95,102] However, not all bacteria capable of breaking down AHLs have all three functional classes of enzymes involved in AHL degradation, which are lactone hydrolases (lactonases), acylases, and oxidoreductases.

The best characterized bacterial lactonases belong to two families: Zn-dependent metallo-β-lactamases (AiiA of *Bacillus* sp. as a prototypical member[93]) and metallo-dependent phosphotriesterases (QsdA from *Rhodococcus erythropolis* as a prototypical member[91]). Recall, that even though mammalian PONs have AHL lactonase activitiy, these calcium-dependent six-bladed β-propeller esterases are structurally and functionally distinct from prokaryotic lactonases.

AiiA (autoiducer inactivating enzyme) from *Bacillus* sp. 240B1 was the first identified AHL-degrading enzyme.[103] Over 100 AiiA sequences from various *Bacillus* spp. have been deposited into the NCBI database within the last 10 years. The presence of the signature Zn-binding motif HxHxDH−H−D−H helped place AiiA homologues within the metallo-β-lactamase superfamily.[104,105] Co-crystallization of the enzyme in the presence of two concentrations (4−25 mM) of *N*-hexanoyl-L-homoserine lactone (C_6-HSL) revealed key features of this enzyme.[105,106] The hydrophilic moiety of the substrate was accommodated within the active-site cavity.[106] Modeling and mutant analyses indicate that Tyr194 and Asp108 are the conserved catalytic residues that participate in lactonolysis.[105,106] In contrast, binding of the hydrophobic moiety was less constrained: depending on the concentration of the substrate, the *N*-acyl side chain was positioned somewhat differently within the Y-shaped catalytic cavity of the enzyme in relation to the zinc atoms.[106] This observation suggested that the catalytic cavity of AiiA is not fully optimized for *N*-acyl chains, even though the lactone moiety was accommodated within the catalytic center.[106] While γ-butyrolactone or L-homoserine lactone were poor substrates for the enzyme, AiiA hydrolyzed AHLs with acyl side chains ranging from 3-OH-C_4 to 3OC_{12}-HSL.[104,105] These results suggest that AiiA has probably evolved to hydrolyze diverse AHLs that *Bacillus* spp. encounter in soils and in the rhizosphere.

A phosphotriesterase-like lactonase, QsdA (AhlA), from *Rhodococcus erythropolis* W2 belongs to the metallo-dependent amidohydrolase superfamily.[107,108] Its homologues are found in most other rhodococci, flavobacteria, agrobacteria, an archeon (*Sulfolobus solfataricus*), and *Mycobacteria*.[107,108] The archeal homologue of QsdA was cocrystallized with C_{10}-homoserine thiolactone (a structural mimic of a bacterial AHL).[109] The *N*-acyl side chain of the substrate mimic fit within the hydrophobic channel formed by loops 7 and 8, with the thiolactone ring over the binuclear metal center and Tyr97 playing an important catalytic role.[109] In hydrolyzing the lactone ring, Tyr97 of *Sso*Pox (the *S. solfataricus* homologue of QsdA) is proposed to play a role similar to that of Tyr194 of AiiA.[109]

AHL acylases are transmembrane β-lactam amidohydrolases that catalyze the hydrolysis of the amide bond of AHLs to release homoserine lactone and the corresponding fatty acid. AHL acylase activity was first discovered in *Variovorax paradoxus*,[95] and the genes responsible for the activity have been cloned from pseudomonads, cyanobacteria, shewanela, and ralstonia.[100,102,110−112] AHL acylases degrade diverse

AHLs, although their effect on the AHLs with long acyl side chains is more pronounced.[110,112]

A cytochrome P450 monooxygenase, CYP102A1 from *Bacillus megaterium* was also shown to degrade AHLs via a pathway that oxidized the acyl side chain of the molecule.[113] This enzyme has broad substrate specificity, capable of oxidizing fatty acids, acyl homoserines, and acyl homoserine lactones. However, acyl homoserines and their lactones were better substrates than the corresponding fatty acids. Both the L- and D-enantiomers of AHLs were the substrates for the enzyme.[113] However only L-enantiomers are synthesized by the bacteria and recognized by other eukaryotic and prokaryotic AHL-degrading enzymes.

What are the fates of the molecules resulting from AHL degradation? In the rhizosphere, homoserine lactones (degradation production of acylase-catalyzed hydrolysis of AHLs) can be further degraded by other bacteria.[114] Homoserine lactone applied to roots of aseptically grown plants was also found to affect stomatal conductance and increase respiration in the host plants,[115] suggesting that plants detect not only bacterial AHLs, but also the compounds that result from AHL degradation by the acylases produced by the rhizosphere microbiota.

The discoveries of AHL-degrading capabilities in bacteria, which do not themselves produce these QS signals, raise important biological questions regarding their ecological functions in host-associated environments. It is unlikely that potential benefits to the plant or animal hosts drove the evolution of AHL-degrading capabilities in the host-associated bacteria that themselves do not make AHLs. Because some soil bacteria, including pectobacteria and soil pseudomonads, produce potent antibiotics in the AHL-mediated manner,[116,117] the ability to inhibit antibiotic synthesis in other cohabitants of the same ecological niche may have favored AHL-degrading strains. This hypothesis is supported by the reports that AHL-degrading isolates inhibit AHL-dependent synthesis of fungicides in biocontrol soil pseudomonads.[90,98] Consistent with this hypothesis, in rhizosphere microcosms, wild-type *Bacillus thuringiensis* was 100−1000 times more competitive than the *aiiA* mutant when the bacteria were co-inoculated with *Pectobacterium carotovorum*,[96] even though in laboratory monocultures wild-type bacteria and their isogenic mutants incapable of degrading AHLs grow with roughly similar rates and reach the same final population densities.[96] An alternative explanation for the reduced fitness of the AHL-ase mutants is also available. AHLs themselves can function as antibiotics and thus directly select for AHL-degrading microbes. For example, in aqueous solutions, β-ketoamide moieties of 3O-AHLs undergo Claisen-like intramolecular alkylation into tetramic acids.[80] Tetramic acids are bacteriocides, which specifically inhibit some Gram-positive bacteria. EC_{50} values range from ~8 to 80 μM, depending on the tetramic acid and Gram-positive organism tested.[80] Therefore, co-inoculation microcosm experiments of the wild-type and *aiiA* mutants with a wild-type *Pectobacterium*[96] leave open the possibilities that AHL themselves or the exoproducts synthesized in the AHL-dependent manner exerted the selective pressure. Regardless of the agent of the selective pressure, the ability to degrade AHLs likely represents an important evolutionary adaptation in the bacteria that do not themselves produce AHL signals.

5.4. Plants and Animals Genetically Engineered To Degrade AHLs and Interfere with QS

Discoveries of QS inhibitors and enzymes capable of degrading bacterial QS signals offered opportunities to test their various biotechnological applications. Because industrial and medicinal applications of QS inhibitors are discussed elsewhere in this QS-themed issue, we will focus on the biotechnological applications of prokaryotic and eukaryotic AHL-degrading enzymes.

The discovery of AHL-degrading enzymes in host-associated bacteria led to the experiments in which the ability of AHL-degrading bacteria to inhibit AHL-dependent virulence in plant and animal pathogens was tested. Co-inoculations of AHL-degrading bacteria with pathogens of plants (like *Pectobacterium carotovorum* and *Agrobacterium tumefaciens*) and invertebrates (like *Vibrio harveyi*) in which AHL QS contributes to the regulation of virulence genes afforded a degree of protection against the pathogens.[90,92,96,99] Furthermore, expression of lactonase and acylase genes in heterologous hosts (typically, plant or animal pathogens in which QS controls virulence genes) led to the reduced accumulation of AHLs in the host and attenuation of its virulence in plant and animal models.[97,103,108,112,118,119]

The encouraging results of the experiments where AHL-degrading bacteria and their enzymes were reasonably successful in reducing virulence of QS pathogens are matched by the published reports that demonstrate that the expression of AHL lactonases in plants and animals confers a similar degree of protection against pathogens. Expression of the *Bacillus* spp. *aiiA* in tobacco and potato reduced accumulation of AHLs in plants infected with the soft rot pathogen *Pectobacterium carotovorum*, and this led to the reduction in the presentation of the soft rot symptoms caused by the pathogen.[93] Similarly, expression of human PON1 in fruit flies obliterated $3OC_{12}$-HSL and protected the animal from *Pseudomonas aeruginosa*.[87] The degree of protection afforded by PON1 was similar to the reduced virulence of the AHL-deficient mutants of *P. aeruginosa*, consistent with the mode of the action of PON1.[87]

Future practical applications of AHL-degrading enzymes in controlling plant or animal pathogens will need to balance host protection from pathogens and avoiding deleterious effects on commensals. This task appears to be more straightforward in animals systems, because none of the characterized commensals of mammals or birds uses self-produced AHLs for gene regulation, while most Gram-negative pathogens of these animals rely on production or perception of AHLs for controlling virulence behaviors. In plant-associated environments, AHL-mediated QS is used extensively by pathogens, commensals, and symbionts. Nonspecific degradation of bacterial AHL signals in plant-associated environments may have unexpected consequences in disrupting the structure or function of the beneficial plant-associated microbiota.

6. Physiological Concentrations of AHLs in the Host Environment

As Table 1 clearly shows, a variety of mammalian and plant cells have been experimentally exposed to many different AHLs at a range of different concentrations. Thus, in order to interpret the physiological relevance of these observations, it is important to know the expected concentration of AHLs in a given environment. Several studies have

tried to quantify the concentrations of AHLs both *in vitro* and *in vivo*, and it is fairly well accepted that the concentration of $3OC_{12}$-HSL present in a *P. aeruginosa* culture, shaking at 37 °C, is 1−10 μM.[25,120] However, $3OC_{12}$-HSL concentrations up to 600 μM have been measured in the media surrounding *P. aeruginosa* biofilms *in vitro*.[121] Considering the considerable difference in the measurements from *in vitro* cultures, what would the expected *in vivo* concentrations be, where degradative enzymes and alterations in pH and temperature abound?

Some of the earliest measurements of AHL concentrations *in situ* came from the very model system in which QS was first observed. Within the light organ of bobtail squid, *Euprymna scolopes,* cell numbers of its symbiont *Vibrio fischeri* can reach 10^{10} cells/mL. Boettcher et al. measured the $3OC_6$-HSL produced by *V. fischeri* within the light organs of squid and determined that the 100 nM concentration detected in the light organs was up to 200 times higher than that observed in culture.[122] The investigators also observed that AHLs not only were found directly around the bacteria but also had penetrated into underlying layers of the squid's epithelia.

AHLs have been detected in the fluid secretions of infected patients in a number of studies, and some have attempted to estimate the concentrations of these chemicals. These studies have predominately examined lung tissue and pulmonary secretions from *P. aeruginosa* infected cystic fibrosis patients,[123−126] where concentrations were measured at picomolar levels in the sputum[123,126] and femtomole per gram levels in the lung tissue.[125] More recently, AHLs have also been detected in infected wounds.[127,128] Rickart et al. surveyed 46 chronic wound clinical isolates belonging to nine different genera and determined that 19.6% produced AHLs when grown in culture.[128] Interestingly, 69.6% of these isolates produced autoinducer-2 (AI-2). AI-2, also known as the "universal autoinducer", is a name given to the family of structurally related compounds produced by both Gram-negative and Gram-positive organisms, whose synthesis is dependent on the LuxS enzyme.[1] The investigators also detected AHLs or AI-2 directly from debrided wound tissue in 21 of 30 clinical samples; however the concentrations of the autoinducers were not measured. Nakagami et al. used an ischemic wound mouse model, to estimate the concentration of $3OC_{12}$-HSL in *P. aeruginosa*-infected wounds.[127] The levels of $3OC_{12}$-HSL remained relatively steady from infection day 3 to 7, only slightly increasing from 0.33 pmol/g on day 3 to 0.49 pmol/g on day 7, and these concentrations represented a linear correlation with the number of bacterial cells.

Taken together, the experimental data produced thus far suggest that the net concentrations of at least $3OC_{12}$-HSL range from femtomolar to nanomolar concentrations in the *P. aeruginosa* infected mammal. However, it is important to note that these estimations represent a net concentration and do not take into account potential spatial and temporal fluctuations. Considering the high levels of $3OC_{12}$-HSL that have been measured near *P. aeruginosa* biofilms *in vitro* (600 μM[121]), it is reasonable to assume that host cells in the immediate vicinity of biofilms will be exposed to much higher concentrations of AHL than those further away. This continuum of AHL may range from "local" micromolar concentrations to more "systemic" picomolar concentrations. Thus, it is important to carefully consider the anticipated bacterial cell density or proximity of host cells to bacteria *in vivo* when interpreting data garnered *in vitro*.

There have been few studies measuring AHL concentrations in the soil environment or on plant surfaces. Bacterial densities and therefore AHL concentrations around plant surfaces and inside plant tissues vary enormously, because colonization occurs in discrete patches[78,129] and is dependent on infection stages of plant−bacterial associations.[130] Using TnpR-based RIVET reporters, Gao and Teplitski[77] measured QS responses in discrete patches (microcolonies) formed on plant root surfaces by *Sinorhizobium meliloti*. It appears that the AHL signal synthesis and responses begin to take place within rhizosphere communities that reach a threshold size, although the minimal "quorate" size of the population was not determined.[77] Gantner and co-workers[44] used reporter strains to monitor bacterial responses to AHLs in the rhizosphere. Reporter strains responding to 20 nM AHLs were shown to be activated even by just a single or very few cells up to 70 μm apart on the root surface. In larger aggregates of bacteria in a tomato rhizosphere, AHL concentrations in micromolar to millimolar range were measured.[44] Dulla and Lindow[78] presented an elegant study using reporter strains to test for QS activation of bacteria colonizing the surface of leaves. The larger colonies were reported to accumulate $\geq$1 μM of AHLs. However, as discussed in this study, the availability of water, which can be very heterogeneous on plant surfaces, played a significant role in determining concentrations and mobility of AHLs on leaf surfaces.[78] Overall, it is very difficult to predict effective AHL concentrations on plant surfaces, and future studies will need to determine how plants deal with detection and response to heterogeneous AHL concentrations around different parts of the plant.

7. Conclusions and Challenges for the Future

While there is little doubt that eukaryotic cells perceive bacterial signals, there are many questions that must be answered before we can fully understand the importance of these signals in bacterial−host interactions. Even though this review focused on host perception of AHLs, there is a vast and diverse array of QSS produced by bacteria, which vary depending on the environmental conditions and substrates available. Thus, it will be important to define what constitutes a true "signal" to bacterial and host cells, determine how many chemically distinct signals can be synthesized by any given bacterial species in any given environment, and determine how modifications to these signals change the messages perceived by the host.

Another fundamental question is whether recognizing bacterial AHLs actually benefits plants and animals. Data demonstrating that *Ulva* zoospores settle and reproduce in response to AHLs[41] and that *Caenorhabditis elegans* may use AHL sensing to locate food sources[131] lend support to this hypothesis. If AHL sensing is indeed advantageous to the host, it could suggest that there has been evolutionary pressure benefiting eukaryotes possessing receptors to sense AHL signals. Thus, one of the most important challenges is to elucidate the host receptors and signal transduction pathways that mediate host perception of bacterial signals. While one mammalian receptor has already been shown to interact with AHLs (PPARγ[6]), further studies are needed to ascertain the physiological relevance of these interactions *in vivo* and determine whether other members of the nuclear hormone family or other distinct families of receptors are

involved in bacterial signal perception. Once receptors and pathways are identified *in vitro*, physiologically relevant *in vivo* models of interkingdom signaling will be needed to elucidate the true contribution of these interactions to pathogenesis or commensalism.

It is clear from the evidence presented here that the effects of AHLs on mammalian cells and the mechanisms mediating them vary greatly dependent on cell type. Therefore another interesting question is whether plant perception and response to AHLs also varies depending on cell type or developmental stage. For example, it is known that many interactions of roots with bacteria are concentrated at the root tip, where exudation rates are high, the root is actively growing, and root hairs are active.[132] While some studies with reporter genes indicate cell specificity of plant responses to AHLs,[43] no systematic analysis of responses in different root or shoot zones has been undertaken thus far. Furthermore it would be interesting to investigate the advantages of developmental changes in response to AHLs that have been observed in some plants. It could be envisaged that changes in root growth or root hair development could enlarge the areas of root surface at which bacteria can interact with plants; that is, root growth responses could actually be an immediate advantage for the bacterial partner and only indirectly influence the plant host.

There are clear differences in how different plant species respond to AHLs. Thus, it will be vital that future studies determine whether these differences stem from the fact that plants have evolved interactions with specific bacterial symbionts and pathogens and whether their AHLs trigger different responses to those of bacteria with which the plants usually do not interact. If so, do AHLs from specific symbionts and pathogens evoke responses in the plant that are beneficial for their interaction with the bacterial partner? Also unexplored is the question of how AHLs move within the plant. There is evidence for movement of AHLs from roots to shoots and that AHL structure, in particular acyl side chain length, determines uptake and movement.[45,50] This could argue for diffusion of AHLs via membranes, but the existence of transporters cannot be excluded.

Lastly, while this review focused on how mammalian and plant cells perceive bacterial QSS, the influence of host signals on bacterial quorum sensing systems is also of significant interest when considering interkingdom signaling. While it has been known for some time that mammalian hormones affect bacterial growth and virulence,[133] it has recently been shown that some hormonal signals can specifically modulate QS in some bacterial species. For instance, the catecholamines epinephrine and norepinephrine can substitute for endogenous QSS and activate the QS-controlled virulence genes in enterohemorrhagic *Escherichia coli* EHEC,[134] and the opioid dynorphin up-regulates the *Pseudomonas* quinolone operon in *P. aeruginosa*.[135] These data suggest that the there may be complementary mechanisms for dual conduit signaling in prokaryotes and eukaryotes.

The "dual conduit" hypothesis is supported by the discovery that vitamin riboflavin (and its derivative lumichrome) can interact with at least some bacterial AHL receptors. These compounds are produced and used as signals by animals, plants, and bacteria, and, apparently, in cross-kingdom signal exchange. Recent genetic advances in the characterization of the synthesis of riboflavin and lumichrome in plants and bacteria are likely to shed light on the actual function of these compounds in the plant−bacterial interactions.

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