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Inter-kingdom signalling: communication between bacteria and their hosts

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Abstract

Microorganisms and their hosts communicate with each other through an array of hormonal signals. This cross-kingdom cell-to-cell signalling involves small molecules, such as hormones that are produced by eukaryotes and hormone-like chemicals that are produced by bacteria. Cell-to-cell signalling between bacteria, usually referred to as quorum sensing, was initially described as a means by which bacteria achieve signalling in microbial communities to coordinate gene expression within a population. Recent evidence shows, however, that quorum-sensing signalling is not restricted to bacterial cell-to-cell communication, but also allows communication between microorganisms and their hosts.

Prokaryotes and eukaryotes have coexisted for millions of years. It is estimated that humans have 10^{13} human cells and 10^{14} bacterial cells (comprising the endogenous bacterial flora). Eukaryotes have a variable relationship with prokaryotes, and these interactions can be either beneficial or detrimental. Humans maintain a symbiotic association with their intestinal microbial flora, which is crucial for nutrient assimilation and development of the innate immune system¹. These mutually beneficial associations are possible because microorganisms and mammals can communicate with each other through various hormone and hormone-like chemical compounds. These signals, however, can be ‘hijacked’ by bacterial pathogens to activate their virulence genes.

The hormonal communication between microorganisms and their hosts, dubbed inter-kingdom signalling, is a recent field of research. This field evolved from the initial observation that bacteria can communicate with each other through hormone-like signals², a process that was later named quorum sensing (QS)³. This field expanded with the realization that these bacterial signals can modulate mammalian cell-signal transduction⁴ and that host hormones can cross-signal with QS signals to modulate bacterial gene expression⁵.

In this Review, we discuss several mechanisms that are used for hormonal communication between micro-organisms and their hosts. Owing to space constraints, we mainly consider pathogenic interactions. We focus primarily on acyl-homoserine lactones (AHLs) and aromatic (autoinducer (AI)-3) signals, because of the wealth of reports that link these signals to inter-kingdom communication, but it is worth noting that bacteria use an array of additional chemical

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DATABASES

Entrez Genome Project: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genomeprj> *Agrobacterium tumefaciens* | *Borrelia burgdorferi* | *Escherichia coli* | *Francisella tularensis* | *Medicago truncatula* | *Providencia stuartii* | *Pseudomonas aeruginosa* | *Salmonella typhi* | *Salmonella typhimurium* | *Sinorhizobium meliloti* | *Vibrio fischeri* | *Vibrio parahaemolyticus*

FURTHER INFORMATION

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molecules to communicate with one another, and it is expected that future research will implicate these in inter-kingdom signalling. After discussing bacteria–mammal and bacteria–plant interactions, we will discuss the evolutionary parallels between host and microorganism signalling systems.

The hormonal signals

There are three broad categories of mammalian hormones: proteins (or peptides), steroids (a subclass of lipidic hormones) and amino-acid derivatives (or amines). The structure of a hormone dictates the location of its receptor. Amine and peptide hormones cannot cross the cell membrane and bind to cell-surface receptors (such as receptor kinases and G-protein-coupled receptors (GPCRs)), whereas steroid hormones can cross plasma membranes and primarily bind to intracellular receptors.

Protein and peptide hormones constitute most of the hormones, contain 3–200 amino acids and are usually post-translationally processed. Peptide hormones include the epidermal growth factor (EGF), insulin and glucagons. Steroid hormones are derived from cholesterol, and amines are synthesized from tyrosine. Amine hormones include the catecholamines adrenaline, noradrenaline (NA) and dopamine⁶. All of these hormones (FIG. 1) are engaged in inter-kingdom signalling with microorganisms.

Communicating through cell-surface receptors

Here, we summarize signalling through mammalian and bacterial extracellular receptors that recognize hormones that do not cross the cellular membrane. Owing to the abundance of reports on inter-kingdom signalling through intracellular receptors, these signalling mechanisms will be discussed separately.

Signalling through receptor kinases

Receptor kinases are cell-surface receptors that possess intrinsic tyrosine- or threonine-kinase activity that becomes activated upon the binding of a hormone to the extracellular amino-terminal region of the receptor. This activation results in the recruitment and phosphorylation of intracellular downstream proteins, which initiates signalling cascades (FIG. 2). One receptor tyrosine kinase that is important for host–microorganism communication is the EGF receptor (EGFR). EGFR exists on the cell surface and is activated by the binding of EGFs, small proteins that are widely used as signals by animal cells⁷. Upon activation by EGF, EGFR undergoes a transition from an inactive monomeric form to an active homodimer, which stimulates the intrinsic intracellular tyrosine-kinase activity of EGFR. Autophosphorylation results in downstream activation and signalling by other proteins, which initiates the signal-transduction cascades, principally the mitogen-activated protein kinase (MAPK), Akt and Jun amino-terminal-kinase (JNK) pathways, that lead to DNA synthesis and cell proliferation. EGFR signalling is required for cell-fate specification, growth and survival at multiple steps of development⁷ (FIG. 2).

The membrane serine protease rhomboid (Rho) regulates EGFR signalling by allowing the secretion of EGFR ligands through the proteolytic activation of EGF from the signal-emitting cells, and provides a link between eukaryotic signalling and host–microorganism communication (TABLE 1). Rho is conserved between eukaryotic and prokaryotic cells. The *Providencia stuartii* Aar protein is a Rho-related protease that can functionally substitute the *Drosophila* Rho^{8,9}. Additionally, eukaryotic Rho can complement a *P. stuartii* aar mutant, which shows that the function of these proteins is conserved between prokaryotes and eukaryotes. Rho is involved in signalling in animal cells, and Aar is also required for the production of a bacterial QS signal^{8,9}. QS in *P. stuartii* represses the expression of an acetyl-

transferase that modifies peptidoglycan (an intrinsic component of the bacterial cell wall)^{10, 11}.

Signalling through GPCRs

Another type of cell-surface receptor that can be activated by hormones is the GPCRs. These receptors have seven transmembrane domains and are coupled to heterotrimeric guanine-binding proteins (G proteins), which consist of an α -, a β - and a γ -subunit. Hormone binding to a GPCR results in a conformational change that induces the receptor to interact with a regulatory G protein; this stimulates the release of GDP in exchange for GTP and results in G-protein activation. The activated G protein (bound to GTP) dissociates from the receptor, which is followed by α - $\beta\gamma$ dissociation, and activates its intracellular target, which can be either an ion channel or an enzyme. GPCR specificity is controlled by the type of G protein with which a GPCR is associated. G proteins can be divided into four families depending on their association with different effector proteins. The signalling pathways of three of these — $G\alpha_s$, $G\alpha_i$ and $G\alpha_q$ — have been extensively studied. The $G\alpha_s$ family activates adenylate cyclase, the $G\alpha_i$ family inhibits adenylate cyclase and the $G\alpha_q$ family activates phospholipase C. Within the largest class of the GPCRs are the mammalian adrenergic receptors, which change their conformation upon binding adrenaline or NA and thereby activate their coupled G protein to initiate a signalling cascade⁶ (FIG. 2).

Bacterial cells do not express adrenergic receptors, but many studies indicate that they respond to adrenaline and/or NA. Most of these studies were conducted in bacteria that inhabit the human gastrointestinal (GI) tract^{5,12–26}, in which, notably, both adrenaline and NA are present. NA is synthesized in the adrenergic neurons of the enteric nervous system²⁷. By contrast, adrenaline is synthesized in the central nervous system and the adrenal medulla; it acts in a systemic manner after being released into the bloodstream, thereby reaching the intestine²⁸. Although during homeostasis NA is probably the pre-dominant signal in the intestine (as it is produced in the enteric nervous system), during stress, adrenaline acts systemically and affects the whole body, including the human GI system²⁸. Both hormones modulate intestinal smooth-muscle contraction, submucosal blood flow and chloride and potassium secretion in the intestine²⁹. Freddolino and colleagues³⁰ reported that the ligand-binding sites for adrenaline and NA in a human adrenergic receptor are similar, and there is evidence to indicate that both adrenaline and NA are recognized by the same receptors and have important biological roles in the human GI tract²⁹. Furthermore, although these hormones are usually found at a nanomolar level in sera, the level of NA in the intestine is in the micromolar range; this is because the enteric nervous system is rich in adrenergic neurons, which have axon terminations that are close to the intestinal epithelia³¹.

Interpreting adrenaline and NA

Bacteria sense and respond to adrenaline and NA to regulate a multitude of phenotypes that range from metabolism to virulence-gene expression.

EHEC as a case study: lessons from a hijacker

NA induces bacterial growth, fimbriae and toxin expression in pathogenic *Escherichia coli*^{12–18}. There are also reports in the literature (albeit conflicting) that imply that NA functions as a siderophore^{17,32}. Recently, NA was implicated in the induced expression of enterobactin and iron uptake in *E. coli*, which suggests that this is the mechanism that is involved in growth induction¹⁸. However, the role of NA in bacterial pathogenesis seems to be more complex, and the line that divides signalling and iron uptake is becoming increasingly blurred; for example, the siderophore pyoverdine in *Pseudomonas aeruginosa* also acts as a signalling molecule³³. It has also been reported that during surgical trauma NA release into

the intestine induces the expression of virulence traits in *P. aeruginosa*, which leads to gut-derived sepsis³⁴. The role of adrenaline and NA signalling in bacterial pathogenesis was solidified by the discovery that both hormones induce the expression of flagella and the type III secretion system (T3SS) in the deadly pathogen enterohaemorrhagic *E. coli* (EHEC) O157:H7. EHEC can sense either the host adrenaline and NA or a bacterial aromatic QS signal, dubbed AI3, to express its virulence traits, which suggests that these host and bacterial signals are interchangeable⁵. EHEC are food-borne pathogens that cause major outbreaks of bloody diarrhoea and haemolytic uraemic syndrome throughout the world³⁵. EHEC colonization of the large intestine causes attaching and effacing (AE) lesions on epithelial cells. These AE lesions are characterized by the destruction of microvilli and pedestal-like structures that ‘cup’ the bacterium^{36–38}. The genes that are involved in the formation of the AE lesion are encoded by the locus of enterocyte effacement (LEE) chromosomal pathogenicity island³⁹. The LEE region encodes a T3SS⁴⁰, an adhesin⁴¹ and its receptor⁴², and effector proteins^{43–47}. The mortality that is associated with EHEC infections stems from the production and release of potent Shiga toxin. Shiga toxin induces cell death in endothelial cells, primarily in the urinary tract, thereby causing haemolytic uraemic syndrome⁴⁸.

EHEC sense three signals to activate their virulence genes — the bacterial signal AI-3, which is produced by several species of bacteria in the normal human GI microbial flora, and adrenaline and NA, both of which are produced by the host^{5,25,49}. Recognition of these three signals is essential for *in vivo* virulence expression, as shown using rabbit and bovine infection models^{23,50}. AI-3, adrenaline and NA are agonistic signals, and responses to all three can be blocked by adrenergic antagonists^{5,23,24}. These signals are sensed by the histidine sensor kinases in the membrane of EHEC that activate a complex regulatory cascade. This cascade culminates in the activation of genes that are necessary for motility, the expression of the LEE (which results in intestinal colonization through the formation of AE lesions) and Shiga-toxin expression^{5,20,23,51}. QseC is one such sensor kinase.

Signal interpretation through QseC and QseB

QseC specifically senses AI-3, adrenaline and NA (to augment its phosphorylation state) by directly binding to these signals. Thus, QseC is a bacterial functional analogue of adrenergic receptors²³. As a *qseC* mutant was attenuated for virulence in two rabbit animal models of infection²³ (*v.S.*, unpublished observations), QseC is essential for full virulence in EHEC.

Typically, sensor kinases constitute two-component systems that act in concert with response regulators. In response to an environmental signal, the sensor autophosphorylates its own conserved histidine residue (FIG. 3). Subsequently, the histidine-bound phosphoryl group of the sensor kinase is transferred onto a specific aspartate residue on the cognate response regulator for activation. The activated response regulator then directly regulates the transcription of its target genes. In bacteria, the two-component system is the major system of signal transduction⁵². Importantly, mammals do not have histidine sensor kinases^{53,54}. Upon sensing AI3, adrenaline or NA, QseC phosphorylates the QseB response regulator, which activates the expression of motility genes²⁰ and itself¹⁹. In addition, QseC plays an important part in the regulation of the LEE genes, iron uptake systems, several adhesins, other two-component systems and Shiga toxin (D.T.H. and *v.S.*, unpublished observations). These *in vitro* and *in vivo* data suggest that QseC has a pivotal role in EHEC pathogenesis and inter-kingdom signalling. The QseC sensor is the first example of a receptor for both a bacterial and a host signal, and, therefore, QseC integrates bacteria–host signalling at the biochemical level.

QseC homologues are present in several other bacterial pathogens, including *Salmonella enterica* serovar Typhi (*S. typhi*), *Salmonella enterica* serovar Typhimurium (*S. typhimurium*), *Vibrio parahaemolyticus* and *Francisella tularensis*. The role of QseC in other bacterial pathogens is beginning to be elucidated. QseC regulates the expression of the PmrAb

two-component system in *S. typhimurium*, which, in turn, regulates virulence gene expression⁵⁵. Also, a recent report demonstrated that a *qseC* mutant was defective in colonization of the swine GI tract⁵⁶. The important role that sensing adrenaline and NA has during *Salmonella* spp. infection and colonization was further highlighted by a report that, in swine, either social stress or stress induced by transportation reactivates subacute *Salmonella* spp. infections and can increase the faecal excretion of these bacteria⁵⁷. *F. tularensis qseC* mutants are attenuated for the infection of mice⁵⁸. Although it has not been associated with QseC, NA has been shown to induce the expression of the *V. parahaemolyticus* T3SS, cytotoxicity and pathogenicity *in vivo*, and these effects were blocked by adrenergic antagonists²⁶. NA has also been shown to regulate the expression of the OspA protein in *Borrelia burgdorferi*, which is important for the survival of *B. burgdorferi* in the tick host and its infection of mice⁵⁹.

Signal interpretation through QseE and QseF

In EHEC, QseC autophosphorylation in response to the three signals that are discussed above can only be blocked by an α -adrenergic antagonist (phentolamine)²³. However, AE lesion formation can be blocked by a β -adrenergic antagonist (propranolol)⁵. These findings suggest that there is a second sensor for these signals. The interaction of AI3, adrenaline and NA with more than one sensor kinase could also provide a 'timing' mechanism during infection, which is desirable, as it would be inefficient for EHEC to express attachment and motility genes simultaneously. In addition to controlling the expression of the motility genes, the LEE and Shiga toxin, QseC also activates the transcription of a two-component system (QseEF), which is involved in AE lesion formation²¹. QseE is a membrane-bound sensor kinase, and QseF is its response regulator. Although the signals that are sensed by this two-component system have not yet been elucidated, the expression of QseE is essential for AE lesion formation by EHEC²¹ and it could constitute a second adrenaline and/or NA sensor in *E. coli*.

AI-3 signalling — talking back to the host?

AI-3 is a bacterial signal that activates the expression of the EHEC virulence genes. AI-3 signalling in EHEC can be substituted by the hormones adrenaline and NA, which suggests that AI-3 might also be involved in inter kingdom signalling⁵. As the host hormones adrenaline and NA signal to EHEC, it would be expected that AI-3 can signal to eukaryotic cells. In the human intestine, enterocytes express the α_2 -adrenergic receptor. This receptor is expressed preferentially in the basolateral compartment of the polarized intestinal cells, but is also found in the brush border of these cells. It is expressed at higher levels in the proximal and transverse colon of the human intestine, which are, coincidentally, the sites of microbial flora and EHEC colonization⁶⁰. A general pathway of α_2 -adrenergic-signal transduction is the depression of intracellular cyclic AMP levels by the inhibition of adenylate cyclase. The α_2 -adrenergic receptor is coupled to both the G_{i2} and G_{i3} signalling proteins, and the activation of G_{i2} is predominantly responsible for the inhibition of adenylate cyclase by α_2 -agonists and natural ligands (adrenaline and NA)⁶¹. Future studies will investigate whether AI3 can inhibit adenylate cyclase through α_2 -adrenergic receptors in enterocytes. Such an inhibition would suggest that AI-3 functions in the human GI tract as a natural α -agonist that is produced by the resident microbial flora. Given that α -adrenergic agonists have been used extensively as inhibitors of chronic diarrhoea⁶², such an activity could suggest an important role for AI-3 in the maintenance of intestinal homeostasis.

The stress response in inter-kingdom signalling

Adaptations to environmental, psychosocial or physical insults in mammals are referred to as stress responses — also termed 'fight or flight' responses. The short-term activation of the stress response involves the synchronized interaction of all aspects of the neuroendocrine

system, and ensures that energy substrates are available to meet the increasing energy demands of the body. Adrenaline and NA are classic stress hormones that have a pivotal role in the stress response⁶. Although they increase a mammal's ability to respond to the environment, it seems that they can be sensed by bacteria, which allows these organisms to gauge the metabolic state of the host and exploit a weakened immune system. In addition to adrenaline and NA, the host releases endogenous opioids, such as dynorphin, during stress. A recent study suggested that the opportunistic pathogen *P. aeruginosa* uses host dynorphin to enhance the expression of its virulence traits; it was also shown that this mammalian stress hormone cross-signals with the *P. aeruginosa* QS signalling system⁶³. Taken together, these studies have shown that there is an intricate connection between host stress signalling, bacterial QS and pathogenesis, which suggests that stress responses, some of the most basic physiological functions in prokaryotic and eukaryotic cells, are central to inter-kingdom communication.

Communicating through intracellular receptors

AHLs and AII are fatty-acid-based signalling molecules (FIG. 1; TABLE 1) that are synthesized by Gram-negative bacteria to coordinate the cell-density-based gene regulation that is known as QS. AHLs mediate bacterial processes by interacting with inducible transcriptional regulators. This process was first characterized in the bioluminescent bacterium *Vibrio fischeri* as a mechanism that regulates light production². The role of AHLs as bacterial signalling molecules has since evolved to include many regulatory functions, including multiple mechanisms of bacterial pathogenesis⁶⁴. These mechanisms are most extensively characterized in *P. aeruginosa*, in which AHLs are required to regulate colonization and persistence during infection^{65,66}.

Lipidic-based or fatty-acid-based signalling mechanisms have also been extensively characterized in eukaryotes. Dozens of lipid-based hormones have been shown to facilitate hundreds of biological functions. These lipid-based molecules include members of the eicosanoid family as well as lipidic and steroid hormones. Lipid-based hormones diffuse freely across the cell membrane and interact with members of the nuclear hormone receptor family to modify transcriptional regulation⁶⁷.

Bacterial AHLs and eukaryotic lipidic signalling molecules are chemically analogous and have comparable modes of action (FIG. 4). This observation led to the hypothesis that bacterial AHLs enter the host cell and mediate the regulation of transcription⁶⁸. A *bona fide* mammalian AHL receptor has not yet been found, but recent studies have shown that the AHL *N*-(3-oxododecanoyl)-L-homoserine lactone (also known as OdDHL) can enter, and function in, mammalian cells⁶⁹.

It has been known for more than a decade that AHLs can elicit responses in mammalian cells. This phenomenon was first observed using respiratory epithelial cells, in which there was a dose-dependent increase in interleukin (IL)-8 in response to purified AHL⁷⁰. Subsequent reports indicated that AHLs have a pleiotropic effect on host cells that depends on cell type, AHL concentration and assay condition. However, several groups have consistently demonstrated what seems to be a biphasic role for AHLs in immunomodulation — low AHL concentrations impair the immune response, whereas high concentrations exacerbate immune function.

Inhibition of the host immune response by AHLs

AHLs were first shown to act as inhibitors of the host immune response in 1998 (REF. 4). In this study, it was reported that OdDHL inhibits lymphocyte proliferation and tumour necrosis factor- α production, and downregulates IL-12 production, in lipopolysaccharide-stimulated macrophages. This assay was used to establish the structural requirements of AHL for immune

inhibition. Acylated L-homoserine lactones that have an 11–13 carbon side chain that contains either a 3-oxo or a 3-hydroxy group have optimal immune suppressive activity⁷¹. Subsequent studies indicated that OdDHL inhibits cytokine production *in vitro* and INF- γ tends to be inhibited more than IL-4. However, *in vivo* experiments were suggestive of nonspecific immune inactivation rather than a T helper 1 (T_H1) response⁷², which supports the finding that OdDHL inhibits the differentiation and proliferation of both T_H1 and T_H2 cells^{73,74}.

AHLs promote apoptosis

Gram-negative bacteria, such as EHEC, enteropathogenic *E. coli* and *Salmonella* spp., secrete effectors to initiate apoptosis and abrogate the innate immune response⁷⁵. Several recent reports indicate that OdDHL also initiates apoptosis, which suggests an additional mechanism of bacteria-mediated apoptosis. OdDHL causes apoptosis in various mammalian cell types, including neutrophils, macrophages, fibroblasts, human vascular endothelial cells and breast carcinoma cells^{76–79}.

The induction of apoptosis by OdDHL was first observed by Tateda and colleagues⁷⁶, who observed a dose-dependent and incubation-time-dependent increase in apoptosis in neutrophils and macrophages upon the addition of OdDHL. Interestingly, this effect was not observed upon the addition of *N*-butanoyl-L-homoserine lactone (also known as C4HSL), another QS molecule⁷⁶. Structure–activity studies correlated the hydrophobicity of the side chain of OdDHL with its apoptotic activity. Several synthetic AHL analogues that had polar and non-polar additions to the distal end of the side chain were tested in these correlation studies; the addition of polar groups abolished apoptotic activity, whereas the addition of bulky hydrophobic groups increased apoptotic activity⁷⁷.

In 2004, the first clue towards understanding the mechanism that underlies AHL-induced apoptosis was provided⁷⁹. In a breast carcinoma cell line, OdDHL diminished signal transduction and activator of transcription 3 (STAT3) activity, but had no effect on mAPK activity, and the pharmacological inhibition of the STAT3 pathway led to the same phenotype as the addition of OdDHL. Furthermore, the expression of a constitutively active STAT3 protein abolished the apoptotic activity of OdDHL⁷⁹.

More recently, calcium signalling was established as a mechanism for OdDHL apoptotic activity⁷⁸. OdDHL increases cytosolic calcium levels in murine fibroblasts and human vascular endothelial cells. Interestingly, the pharmacological inhibition of calcium signalling pathways abolishes the apoptotic effect of OdDHL, but the immunomodulatory effects of OdDHL remain intact⁷⁸.

AHLs elicit proinflammatory effects

Following the discovery that IL8 levels increase in respiratory epithelial cells upon the addition of OdDHL, a series of studies that documented numerous proinflammatory pathways that are affected by OdDHLs were published⁸⁰. The first of these studies characterized the potential mechanism by which OdDHL could increase the production of IL-8. The transcription factors nuclear factor (NF)- κ B and AP2 are required for maximal induction of IL-8 by OdDHL⁸⁰. Furthermore, if the mAPK pathway is inhibited, OdDHL stimulation of both IL-8 and NF- κ B is subdued⁸¹. However, OdDHL also induces neutrophil chemotaxis in an IL-8-independent manner⁸⁰.

Subsequent studies examined the effect of OdDHL injection into the skin of mice. Injection resulted in increased mRNAs for the cytokines IL-1 α and IL-6 as well as for the chemokines macrophage inflammatory protein 2 (mIP2), mIP1- β , interferon- γ inducible protein 10 and T-cell activation gene 3 (REF. 82). OdDHL also activated T-cells to produce INF- γ ⁸².

Acting through the NF- κ B pathway, OdDHL increases the expression of cyclooxygenase 2 (COX2); an enzyme that is responsible for the synthesis of prostaglandin (an eicosanoid)⁸³. Increased levels of membrane-associated prostaglandin E (PGE) synthase, as well as its product, PGE2, indicate that OdDHLs can act as potent activators of the lipid-mediated immune response. Prostaglandin metabolites, which are similar to OdDHL in structure and their chemical characteristics, have the same COX2 activation phenotype as OdDHLs; this indicates functionality through a common receptor⁸³.

On the forefront: novel roles for AHLs

Rumbaugh⁸⁴ has recently undertaken an unbiased approach to understanding the role of AHLs in mammalian cells. In this study, a microarray analysis of mouse fibro-blasts treated with OdDHL or C4-HSL was performed. Treatment with OdDHL and C4-HSL using Affymetrix GeneChips elicited significant changes in murine genes of 10% and 8%, respectively. Most of these differentially regulated genes exhibited AI specificity (regulated by either OdDHL or C4-HSL). Intriguingly, whereas several of these differentially regulated genes were from categories of previously discovered AHL targets, such as immune modulation and apoptosis, most of the differentially regulated genes were new AHL targets. Therefore, AHLs probably act on multiple mammalian pathways, and these interactions are probably not pathogenic in nature.

Mammals fight back: destroying AHLs

Recent studies have found cytoplasmic factors that degrade OdDHL, called paraoxonases (PONs), in mammalian cells^{85,86}. PONs are involved in the detoxification of many organophosphates and act as lactonases^{85,86}. Although it is possible that these functions evolved only to subvert the QS capabilities of an invading bacterium, this seems unlikely given that QS typically occurs outside the cell. It is more likely that these molecules evolved to prevent OdDHLs, which can infiltrate mammalian cells, from interfering with cellular functions.

In 2004, Chun and colleagues⁸⁶ discovered that human airway epithelia inactivate OdDHL through an unknown mechanism, and activity is cell associated (that is, it is not a secreted factor). The likely candidates for the factor were subsequently reduced to the paraoxonase family proteins PON1, PON2 and PON3 (REFS 87,88). Although serum PON1 subverted *P. aeruginosa* QS and biofilm formation *in vitro*, *Pon1*-knockout mice were protected in a *P. aeruginosa* infection model. PON2 was eventually determined to be the most likely candidate for OdDHL degradation in mammals⁸⁹. Tracheal epithelial-cell lysates from a *Pon2*-knockout mouse had impaired OdDHL-degrading activity, whereas overexpression of *Pon2* enhanced OdDHL degradation⁹⁰. Lysates from *Pon1*- and *Pon3*-knockout mice did not have impaired OdDHL-degrading activity⁹⁰.

AHL-binding domains share similarities with PAS and GAF domains

Recent studies into the physical nature of AHL-binding domains have revealed structural similarities to PAS and GAF domains. The crystallization of TraR, an *Agrobacterium tumefaciens* QS protein, facilitated the first structural insights into the AHL-binding domain. The three-dimensional structure of TraR indicated that it bound to its ligand (*N*-(3-oxo-octanoyl)-L-homoserine lactone) it shared structural similarities with the evolutionarily conserved PAS and GAF domains^{91,92}. The recently solved structures of two other bacterial proteins that contain AHL-binding domains, LasR and SdiA, also showed that they share structural similarities with PAS and GAF domains^{93–96}.

The PAS and GAF domains are also found in the mammalian aryl hydrocarbon receptor (AhR), which shares functional similarities with bacterial AHL-induced transcription factors. AhR is a ligand-induced transcription factor that enters the nucleus and acts as a transcriptional regulator upon engagement by a hydrophobic molecule⁹⁷. Other examples of mammalian PAS and GAF-domain-containing proteins include the neuronal carbon monoxide sensor NPAS2 (neuronal PAS domain protein 2), which regulates the mammalian circadian clock⁹⁸, and HIF1 (hypoxia-inducible factor 1), which regulates the hypoxic response⁹⁷.

Pathogens sense the host immune system

The host immune system can use bacterial signals to gauge, and respond to, bacterial infections, but bacteria have also evolved the ability to sense and manipulate the host immune system. The *P. aeruginosa* OprF surface protein binds host interferon- γ and initiates a signalling cascade in the bacterial cell that reprogrammes gene expression. It has been proposed that by this mechanism pathogens can detect if they have triggered the host innate immune response⁹⁹. In *S. typhimurium*, the PhoQ sensor kinase directly binds, and is activated by, host antimicrobial peptides. PhoQ then promotes the expression of virulence genes through a phosphorelay cascade¹⁰⁰. These findings represent yet another molecular mechanism by which bacteria can sense small innate immune molecules and modulate virulence-gene expression.

Bacteria–plant communications

Many plant-specific pathogenic bacteria require QS to successfully mediate host infection¹⁰¹. However, plants and algae have evolved multiple mechanisms to interpret these QS signals and initiate defensive responses¹⁰¹. Nanomolar concentrations of AHLs cause substantial changes in protein expression in *Medicago truncatula* (alfalfa). Peptide-mass fingerprinting of 100 root proteins that were affected by AHLs revealed that 25% of these proteins had functions in host defence, whereas the remainder had roles in primary metabolism, plant-hormone responses, transcriptional regulation, protein processing and cytoskeletal activity¹⁰². Additionally, plants can respond differently to unique AHLs in a tissue-specific manner¹⁰². For example, during microarray analysis, the treatment of tomato-plant shoots with AHL resulted in the upregulation of multiple defence mechanisms¹⁰³.

As a defence against pathogenic bacteria, plants and algae have mastered the art of AHL mimicry; they secrete hormones that mimic bacterial QS signals, which leads to confusion among the signalling bacteria. Interestingly, the secretion of these mimic compounds seems to be regulated by bacterial AHLs. For example, *M. truncatula* secretes more AHL mimics after bacterial AHL treatment¹⁰². Several groups have isolated and identified individual inhibitory mimic compounds. The most extensively studied are the halogenated furanones that are produced by the marine alga *Delisea pulchra*. *D. pulchra* furanones are structurally similar to bacterial AHLs. Furanones have been found to specifically inhibit AHL-regulated mechanisms in multiple bacterial species. In their natural marine environment, *D. pulchra* uses halogenated furanones to control the structure of the natural bacterial communities that are on the algal surface. The exact mechanism of action is unknown, but it is thought to increase the turnover of bacterial AHL-induced transcription factors¹⁰⁴.

A detailed inhibitory mechanism has also been described in higher plants that have been infected with *A. tumefaciens*, the bacterium that causes crown gall tumours. Formation of these tumours requires plant-tissue injury. In the event of an injury, the plant facilitates the accumulation of γ -amino butyric acid (GABA; also an animal neurotransmitter) near the wound. GABA activates *A. tumefaciens* AttM lactonase, which causes the bacterium to destroy its own QS signal¹⁰⁵. In addition, L-canavanine, produced by alfalfa, inhibits QS in the plant symbiont *Sinorhizobium meliloti*¹⁰⁶. Furthermore, two compounds that were isolated from garlic inhibited QS in a LuxR reporter assay¹⁰⁷.

However, bacteria–plant communication is not only necessary as a defence mechanism. One of the best-studied inter-kingdom signalling mechanisms is found in communications between *Rhizobium* spp. soil bacteria and their symbiotic legume hosts. In this system, legumes produce flavanoids, which are ligands for the bacterial NodD protein. Once activated, NodD activates the expression of other bacterial genes that encode Nod factors, which, in turn, activate plant root receptors and thereby initiate nodulation¹⁰⁸.

Evolution of cell–cell signalling

Recently, an intriguing opinion was written by Iyer and colleagues¹⁰⁹ that examined the evolution of low-molecular-mass first-messenger metabolic pathways, including the synthesis of amino acids, amino-acid derivatives (NA, adrenaline, dopamine, serotonin and melatonin), nucleosides, histamine, lipid- and fatty-acid derivatives (acetylcholine) and inorganic molecules, such as carbon monoxide and nitric oxide¹⁰⁹. They proposed that the evolution of cell–cell signalling in mammals relies more on late horizontal gene transfer from bacteria to animals than on vertical inheritance. This hypothesis was driven by the observation that the enzyme that regulates vertebrate melatonin biosynthesis, arylalkylamine *N*-acetyl-transferase, is encoded in bacteria, yeast and vertebrates, but not in plants, worms or flies¹¹⁰. They suggested that this pattern is consistent with an evolutionary mechanism that involves horizontal gene transfer and multiple gene losses. Of 17 major enzymes that are dedicated to messenger metabolism, only 2 are present in the 3 major crown-group lineages of eukaryotes (animals, plants and fungi) and in bacteria. Sixteen of these enzymes are present in animals and bacteria, and none of these enzymes is present in archaea, which share multiple vertically inherited core-gene sets with eukaryotes¹⁰⁹. Additionally, the scattered distribution of these enzymes does not follow the distribution pattern of central metabolic genes, which generally are represented in all eukarya, bacteria and archaea. Interestingly, another enzyme that is involved in melatonin production, hydroxyindole *O*-methyltransferase, is found only in bacteria and vertebrates. Horizontal gene transfer indicates that bacteria and mammals have the same intrinsic ability to produce multiple mammalian signalling compounds. This argument substantiates a growing body of evidence⁸⁴ that bacteria produce small molecules for bacteria–bacteria communication that also function directly in bacteria–host communication.

Box 1 | Questions for future research

- Do bacteria peptide hormones influence host signalling, and do host peptide hormones influence bacterial quorum sensing?
- Does autoinducer-3 signal to host cells, and if so, is this signalling adrenergic dependent?
- Are there more bacterial adrenergic receptors?
- What are the receptors for acyl-homoserine lactones in mammalian cells?
- Do bacterial cells sense steroid and lipidic mammalian hormones?

Conclusion

The inter-kingdom signalling field is in its infancy, but a growing body of work has demonstrated that inter-kingdom signalling has broad implications to evolution and for mammalian and plant health. Cross-signalling between bacterial AIs and host hormones are at the core of microbial–host communication. However, there are several questions to be answered (BOX 1), including whether bacterial peptide hormones and AI-3 also induce host signalling, and whether lipidic and/or steroid host hormones signal to bacteria. most of the

receptors for these inter-kingdom signals in bacteria and their host also remain to be identified. Future studies in this field will enhance our understanding of the signalling networks that have driven the co-evolution of prokaryotes and eukaryotes. Furthermore, future studies may help us to exploit these communication systems for the design of novel therapeutics to combat bacterial infections and enhance host immune defences.

Glossary

Autoinducer, A bacterial hormone-like signalling molecule.; Enteric nervous system, A nervous system that innervates the gastrointestinal tract.; Siderophore, A small organic molecule that is produced by bacteria to sequester iron.; Type III secretion system, A specialized syringe-like secretion system that is used to inject bacterial effectors into host cells.; Haemolytic uraemic syndrome, A complication, caused mostly by Shiga toxin, that can cause the kidneys to shut down and results in high morbidity and mortality.; Enterocyte, An epithelial cell in the intestine.; Eicosanoid family, A lipid-based signalling molecule that is best known for its control of the immune response. Prostaglandins are part of the eicosanoid family.; T helper 1 (T_H1) response, The actions of CD4 helper T lymphocytes can be summarized by two pathways, T_H1 and T_H2, on the basis of the cytokines that they produce and their effector functions. During the T_H1 response, T helper lymphocytes principally secrete interferon- γ to activate phagocyte-mediated defence, which typically involves intracellular microorganisms.; Paraoxonase family, A three-member gene family that consists of PON1, PON2 and PON3. Mammalian biological functions of paraoxonases remain elusive. However, possible functions include: protection from organophosphate poisoning; a protective role in vascular disease through lipoprotein lipid oxidation; and the limitation of bacterial infection through potential lactonase activity.; PAS and GAF domain, A ubiquitous protein motif that is conserved in prokaryotes and eukaryotes..

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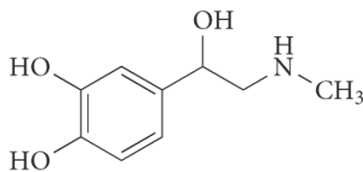
Acknowledgements

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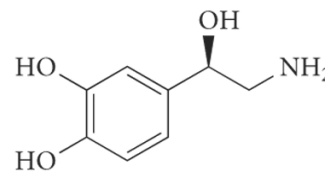
Amines

Structure unknown,
aminated aromatic
compound

AI-3
(bacteria)

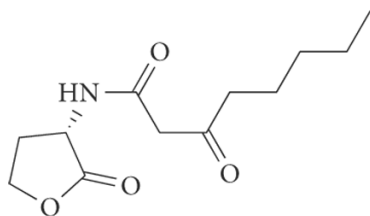


Adrenaline
(host)

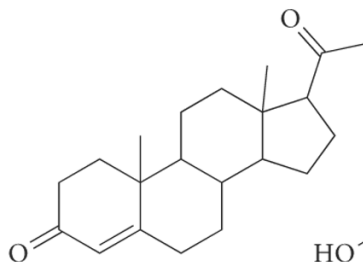


Noradrenaline
(host)

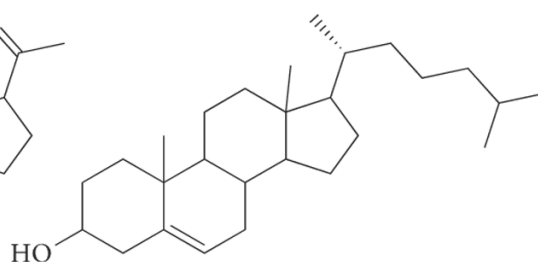
Lipidic steroids



AHL
N-3-oxooctanoyl-L-
homoserine lactone
(bacteria)



Progesterone
(host)



Cholesterol
(host)

Figure 1. Chemical structures of bacterial and host signals

The bacterial signal autoinducer (AI)-3 is an aromatic aminated signal; its final structure is still unknown. Because AI-3 is, to a certain degree, hydrophobic, it is not thought to be able to cross the cell membrane. The bacterial signal acyl homoserine lactone (AHL) is composed of a conserved homoserine ring and a variable acyl chain, and usually can cross the cell membrane. The host hormones adrenaline and noradrenaline are catecholamines that are synthesized from tyrosine and usually do not cross the cell membrane. The host signals progesterone and cholesterol are two examples of lipid host hormones that can cross the cell membrane and bind intracellular receptors.

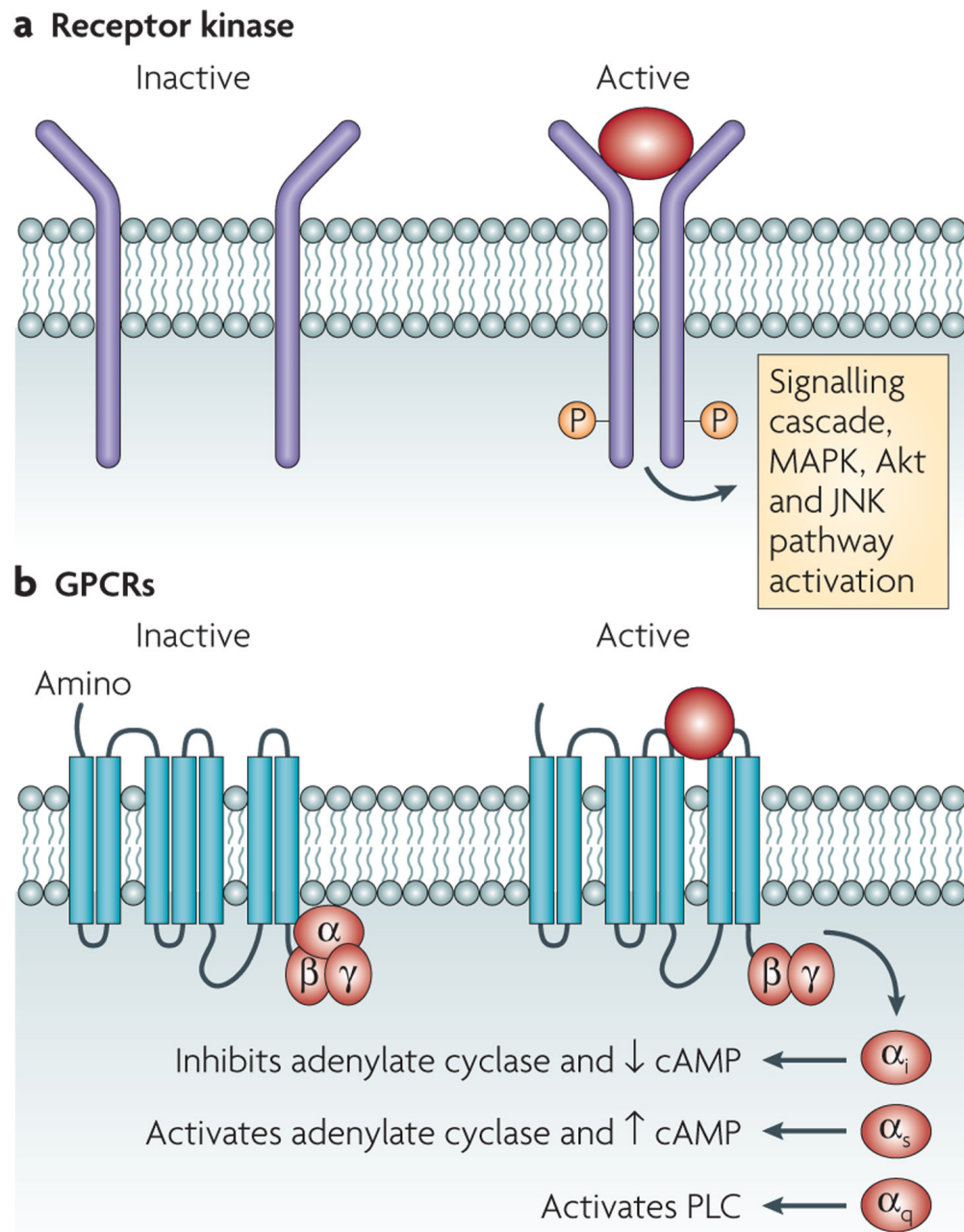


Figure 2. Mammalian signalling through membrane Receptors

a | Membrane receptor kinases dimerize and autophosphorylate a tyrosine or threonine residue upon binding to a hormone. They then initiate a phosphorelay signalling cascade in the cell. These cascades can include the mitogen-activated protein kinase (MAPK), Akt and Jun amino-terminal-kinase (JNK) pathways, and often lead to DNA synthesis and cell proliferation. **b** | G-protein-coupled receptors (GPCRs) are receptors that are coupled to guanine-binding proteins (G proteins) when they are inactive. G proteins consist of an α -, a β - and a γ -subunit. If a GPCR binds its signal (such as adrenaline or noradrenaline (NA)), the α -subunit of the G protein is uncoupled and exerts its effect. Different families of the α -subunit associate with different effectors and exert different effects. The α_i inhibits adenylate cyclase and diminishes

the levels of intracellular cyclic AMP (cAMP), the α_s activates adenylate cyclase to increase the levels of cAMP and the α_q activates phospholipase C (PLC).

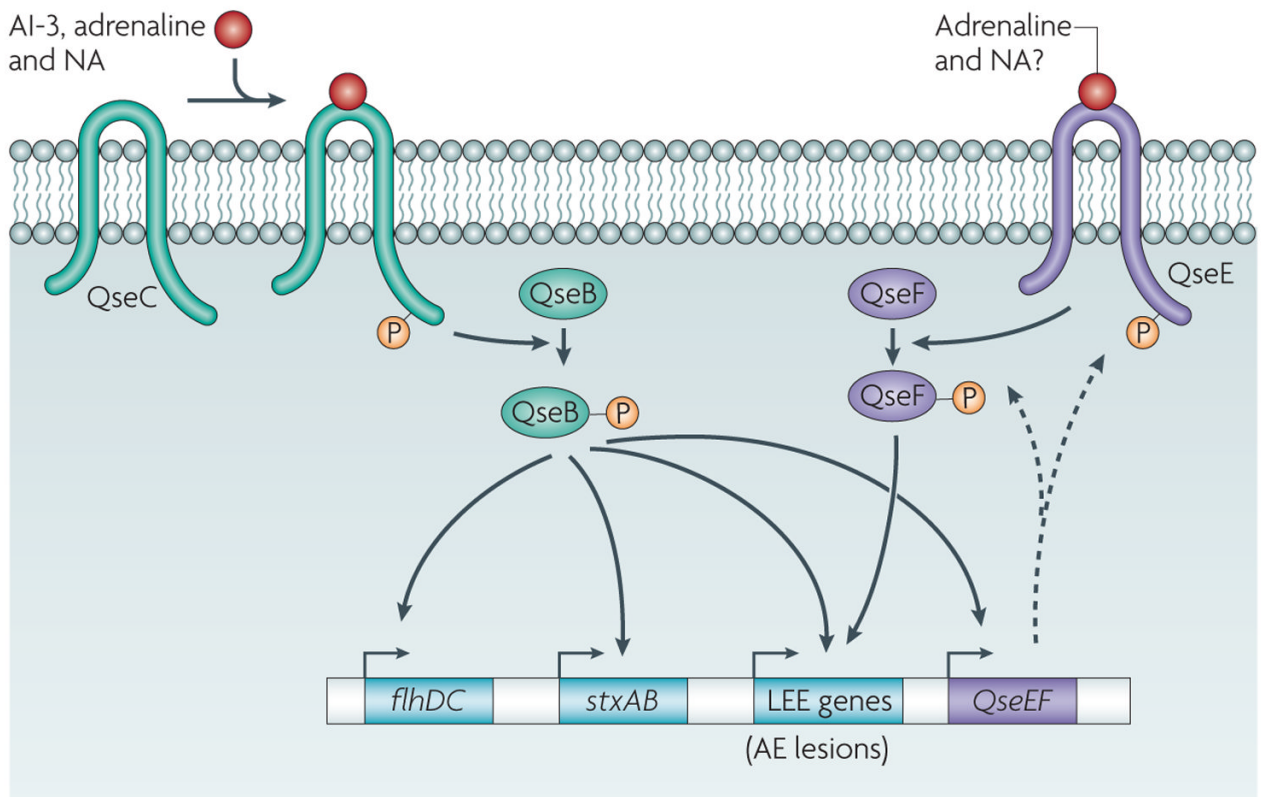


Figure 3. Adrenergic sensing in enterohaemorrhagic *Escherichia coli*

Autoinducer (AI)-3, adrenaline and noradrenaline (NA) bind the bacterial membrane receptor QseC, which results in its autophosphorylation. QseC then phosphorylates its response regulator QseB and initiates a complex phosphorelay signalling cascade that activates the expression of a second two-component system (QseEF), the locus of enterocyte effacement (LEE) genes (which encode various proteins, including the components of a type III secretion system that are involved in attaching and effacing (AE) lesion formation), the motility genes (*flhDC*) and Shiga toxin (*stxAB*). The QseEF two-component system is also involved in the expression of the LEE genes, and although its activators have not yet been elucidated, it is possible that it senses adrenaline and/or NA.

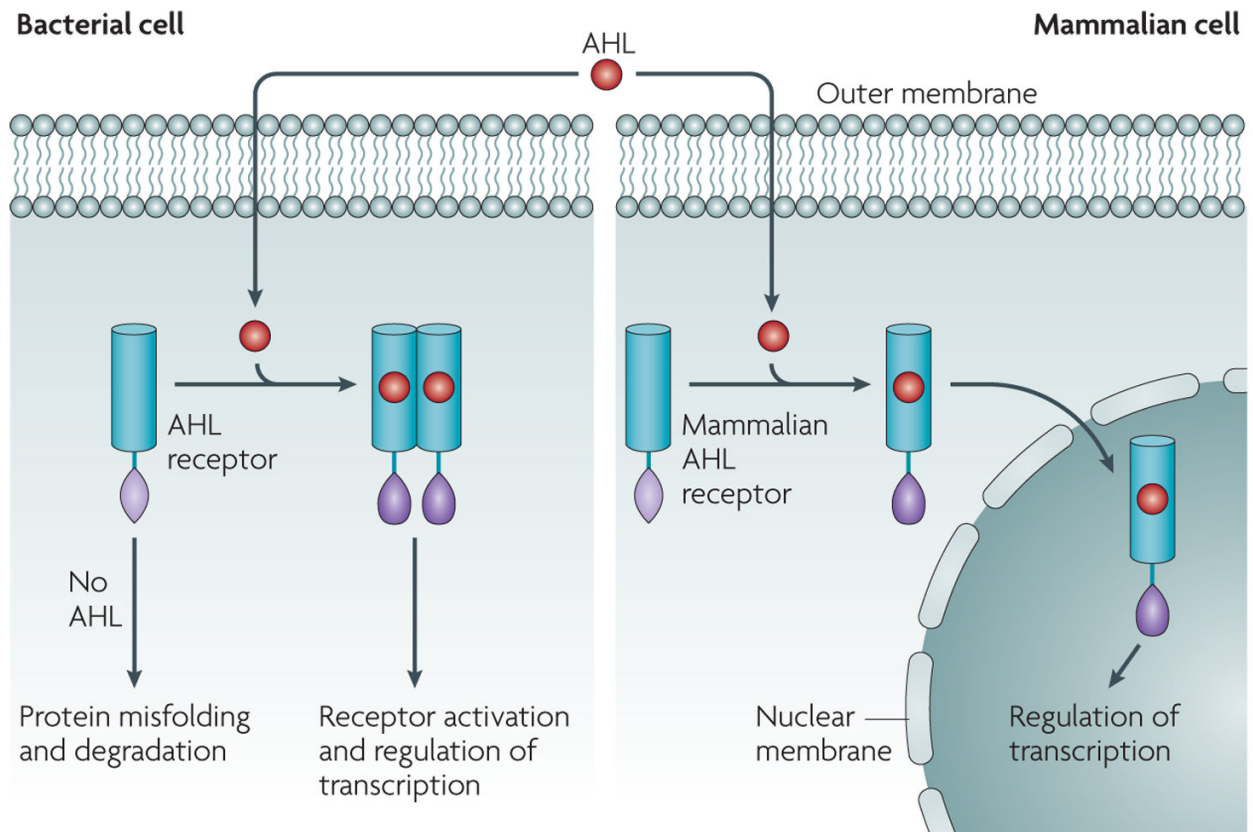


Figure 4. AHL inter-kingdom signaling

In bacteria, acyl homoserine lactones (AHLs) cross the cell membrane and interact with cytoplasmic receptors of the LuxR family. Binding the AHL to the LuxR-type receptor allows proper folding of this protein, which allows the receptor to dimerize and bind to its target sequence on DNA to regulate gene expression. In the absence of signal, LuxR-type proteins misfold and are targeted for degradation. In mammalian cells, AHLs also gain access to the cytoplasm by crossing the plasma membrane. The identity of the mammalian receptor (or receptors) for AHLs in mammalian cells is unknown. However, if there are intracellular receptors, it is proposed that the interaction with the AHL ligand activates these receptors and thereby allows their transportation into the nucleus, where they could control gene expression.

Table 1

Hormonal signals, receptors and biological functions

Signal	Prokaryotic receptor	Prokaryotic function	Eukaryotic receptor	Eukaryotic function	refs
<i>Prokaryotic</i>					
<i>Providencia stuartii</i> autoinducer (AI)	Unknown	Peptidoglycan modifications	Unknown	Unknown	10,11
AI-3	QseC	Type III secretion system (T3SS) activation, motility, toxin expression, iron uptake and virulence	Unknown	Unknown	5,19–25
Acyl homoserine lactones	LuxR, TraR, LasR and others	Virulence, T3SS regulation, biofilm formation, motility, antibiotic production and others	Unknown	Immunomodulation, intracellular calcium signalling and apoptosis	65–94
<i>Eukaryotic</i>					
Adrenaline and noradrenaline	QseC	T3SS activation, motility, toxin expression, iron uptake, virulence, growth and quorum sensing (QS)	Adrenergic receptors	Cyclic AMP levels, phospholipase C activation, stress, cell proliferation, enzyme production and ion channels	5,6 12–25
Peptide (epidermal growth factor (EGF))	Unknown	Unknown	EGF receptor	Cell proliferation, growth and development	6,8
Dynorphin	Unknown	QS and virulence	μ dynorphin opiate receptor	Stress responses	63
Steroid hormones	Unknown	Unknown	Nuclear receptors	Reproduction and regulated metabolism	6