MAKING 'SENSE' OF METABOLISM: AUTOINDUCER-2, LuxS AND PATHOGENIC BACTERIA

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Abstract | Bacteria exploit many mechanisms to communicate with each other and their surroundings. Mechanisms using small diffusible signals to coordinate behaviour with cell density (quorum sensing) frequently contribute to pathogenicity. However, pathogens must also be able to acquire nutrients and replicate to successfully invade their host. One quorum-sensing system, based on the possession of LuxS, bears the unique feature of contributing directly to metabolism, and therefore has the potential to influence both gene regulation and bacterial fitness. Here, we discuss the influence that LuxS and its product, autoinducer-2, have on virulence, relating the current evidence to the preferred niche of the pathogen and the underlying mechanisms involved.

BIOFILM
Complex population of organisms on a surface.

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In most environmental niches, bacteria exist in complex communities of single or multiple species that develop on abiotic (for example, rocks) or biotic (for example, host mucosal tissues) surfaces[1–3] rather than as single, planktonic cells suspended in liquid. As such niches are diverse, microorganisms depend on their capacity to sense the local environmental conditions and adapt by regulating the expression of specific genes. This is particularly vital for pathogenic bacteria, which encounter a range of habitats in a host. Consequently, bacteria have developed several highly sophisticated mechanisms to gather, process and transduce environmental information such as pH, temperature, nutrient availability, osmolarity and cell population density. Many different signal transduction mechanisms in bacteria control the synthesis of colonization factors, toxins and tissue-degrading enzymes, which might only be necessary in specific host compartments and detrimental to the bacterium in others[4].

Many bacteria secrete small, diffusible, signalling molecules. It is generally assumed that these molecules are used for a process termed 'quorum sensing', the phenomenon whereby the accumulation of specific, diffusible, low-molecular-weight signal molecules (or 'autoinducers') enables bacteria to sense when the minimal number, or 'quorum', of bacteria has been achieved for a concerted response to be initiated[5–7]. Both Gram-negative and Gram-positive pathogens are known to use autoinducer molecules to coordinate expression of genes crucial for virulence and survival. Delaying virulence factor production until a certain population density is reached might allow for a host to be overwhelmed before its innate immune response is fully activated. In addition, this process might enable bacteria to function in concert with the characteristics of a multicellular organism by acquiring an organization such as a BIOFILM, which can aid either pathogenesis or the establishment of specific symbiotic associations with eukaryotic hosts[8]. The production of autoinducers might also facilitate the detection of a diffusion barrier or compartment boundary[9,10].

Among the many different mechanisms for quorum sensing that have evolved[7], the most-characterized quorum-sensing systems involve the production of

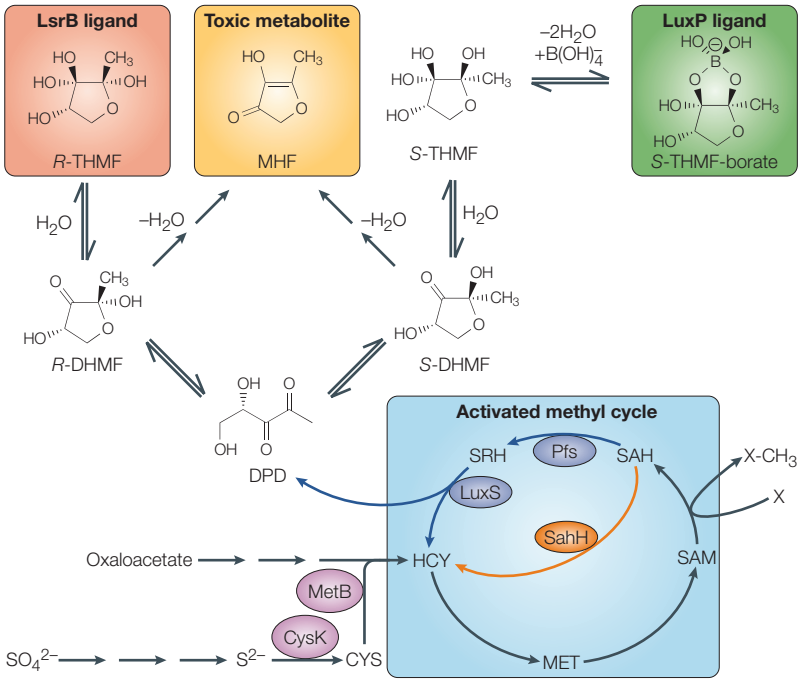

Figure 1 | **Chemical interconversions of molecules in relation to the activated methyl cycle.** The reactions that generate homocysteine (HCY) from (i) sulphate (SO_4^{2-}) through sulphide (S^{2-}), and (ii) oxaloacetate (OA), plus some of the enzymes involved (MetB and CysK) are shown feeding into the boxed reactions that comprise the activated methyl cycle (shaded in light blue). The latter involves the formation of methione (MET) and the subsequent conversion to *S*-adenosylmethionine (SAM). The activated methyl group (CH_3) of SAM is used for methylation of RNA, DNA, certain metabolites and proteins (X), leading to the formation of the toxic metabolite *S*-adenosylhomocysteine (SAH). SAH is then removed and the cycle completed by one of two routes, depending on the organism. One route involves the one-step conversion of SAH to HCY by SAH hydrolase (SahH), the other requires the production of *S*-ribosylhomocysteine (SRH) by Pfs and then the generation of HCY from this by LuxS, which simultaneously generates 4,5-dihydroxy-2,3-pentanedione (DPD). There is spontaneous cyclization of DPD to either the *R* or *S* form of 2,4-dihydroxy-2-methyldihydro-3-furanone (DHMF). *R*-DHMF can then undergo hydration to form the molecule which was co-crystalized with LsrB, (*2R,4S*)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran (*R*-THMF, red box). Alternatively, hydrolysis can occur to create 4-hydroxy-5-methyl-3(*2H*)-furanone (MHF, yellow box). MHF can also be formed through hydrolysis of *S*-DHMF, which can additionally undergo hydration to form *S*-THMF and subsequently form a diester with boric acid to generate the ligand found in complex with LuxP (green box). Where known, the reversible nature of interconversions are indicated by the double arrows. Based on data from REFS 19,28,31.

ACTIVATED METHYL CYCLE
Metabolic cycle which generates the methyl donor *S*-adenosyl-L-methionine and recycles methionine through *S*-adenosyl homocysteine and homocysteine. *S*-adenosyl-L-methionine provides activated methyl groups for use in the methylation of proteins, RNA, DNA and certain metabolites.

S-ADENOSYL-L-METHIONINE
Important methyl donor in the cell.

S-ADENOSYL-L-HOMOCYSTEINE
Produced from *S*-adenosyl-L-methionine following the release of methyl groups by methyltransferases.

N-acyl-L-homoserine lactones (AHLs) by Gram-negative bacteria[6,11,12]. AHLs are usually produced by a protein homologous to LuxI, but can be generated by other enzymes[13–16]. Signalling through the production of post-translationally modified peptides is confined to Gram-positive bacteria[17].

The only quorum-sensing mechanism shared by both Gram-positive and Gram-negative bacteria involves the production of autoinducer 2 (AI-2) by the enzyme LuxS[8,18]. This pathway is found in over 55 species, leading to the suggestion that AI-2 is a universal language for interspecies communication[18]. However, LuxS has an alternative role in the cell, in which it functions as an integral component of the ACTIVATED METHYL CYCLE (AMC)[19]. This link could also provide an explanation for the widespread conservation of *luxS*, although further investigation is required to confirm this possibility.

Here, we discuss the physiology of AI-2 production and sensing by bacteria, and the relationship between LuxS and the AMC. Furthermore, we review the recent data on the impact of LuxS on the pathogenesis of human infections.

LuxS and AI-2

Formation of AI-2. AI-2 is a byproduct of the AMC (FIG. 1), which recycles *S*-ADENOSYL-L-METHIONINE (SAM), the main methyl donor in eubacterial, archaebacterial and eukaryotic cells. As part of the AMC, SAM is converted to *S*-ADENOSYL-L-HOMOCYSTEINE (SAH), which is subsequently detoxified by the Pfs enzyme (also called 5'-methylthioadenosine/*S*-adenosylhomocysteine nucleosidase) to generate adenine and the sole intracellular source of the substrate of LuxS, *S*-RIBOSYL-HOMOCYSTEINE (SRH). LuxS then produces the precursor of AI-2, 4,5-DIHYDROXY-2,3-PENTANEDIONE (DPD), during the conversion of SRH to HOMOCYSTEINE (HCY)[8,20]. Many organisms, in particular eukaryotes, replace this two-step process with a single enzyme, SAH hydrolase (SahH), which converts SAH to homocysteine without producing AI-2 (REFS 20,21).

Biochemistry of the reaction catalysed by LuxS. The available crystal structures of LuxS homologues[22–24] depict a homodimer with a fold that is unique to a small family of enzymes with related functions. Two identical active sites are formed at the dimer interface by residues from both subunits contained within the invariant HXXEH motif, where an Fe^{2+} ion is tetrahedrally coordinated. To achieve the predicted chemical conversion, it is proposed that a series of proton-transfer reactions occurs, catalysed by the Fe^{2+} ion[25] and two residues of LuxS[26]. These properties identify LuxS as the previously described ribosylhomocysteine-cleavage enzyme[27,28] shown to be responsible for DPD formation in *Escherichia coli* (for more detailed overviews of LuxS structure and catalysis see REFS 19,29).

The chemical nature of AI-2. Although DPD is the molecule synthesized alongside HCY by LuxS, AI-2 activity is associated with cyclic derivatives of this molecule that can be generated spontaneously[30,31]. The chemical structures first attributed to AI-2 activity were furanone derivatives (particularly 4-HYDROXY-5-METHYL-3(2H)-FURANONE, MHF), by virtue of their ability to induce bioluminescence in the *Vibrio harveyi* bioassay[20,32]. Although it can activate the *V. harveyi* biosensor and is the main product present in methanol extracts of the *in vitro* AI-2 synthetic reaction mixture, MHF (which can be derived from DPD, see FIG. 1) showed lower activity than AI-2, which indicates that there is at least one other molecule in LuxS *in vitro* reaction mixtures with a higher activity than MHF[19,20,32]. The finding that more than one molecule can behave as AI-2 initiated the use of 'AI-2' as a collective term, a practise that was required after the discovery of further DPD-derived molecules found in complex with two AI-2-binding proteins following co-crystalization.

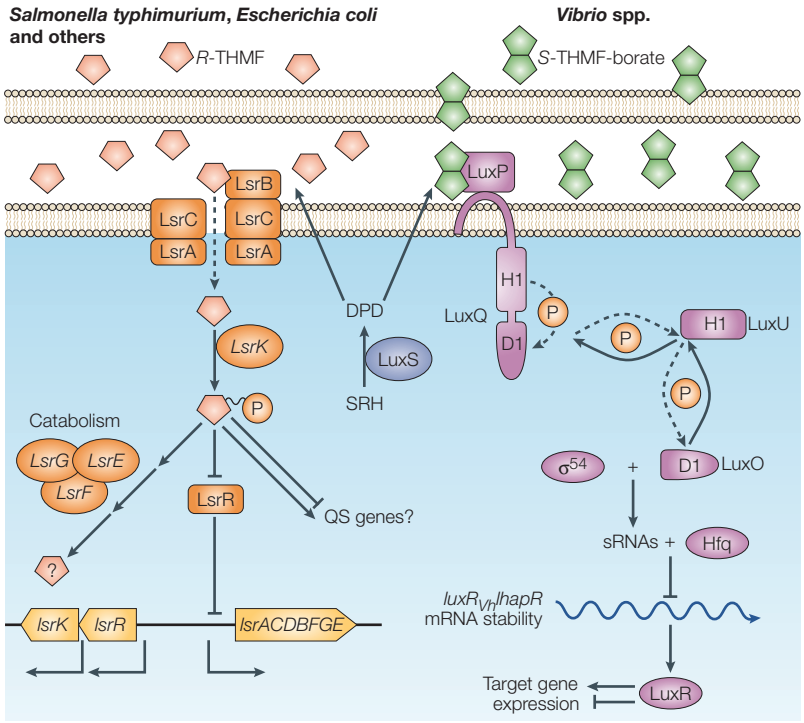

Figure 2 | **Current schemes of autoinducer 2 response.** LuxS (depicted as a blue oval) converts *S*-ribosylhomocysteine (SRH) to the precursor of autoinducer 2 (AI-2), 4,5,-dihydroxy-2,3-pentanedione (DPD), in the cytoplasm. DPD then undergoes spontaneous cyclization and export to the culture supernatant. Depending on the bacteria, response to AI-2 can follow one of the two currently identified routes. In one group of bacteria exemplified by *Salmonella* (shown on the left), the cyclic derivative of DPD, (*2R,4S*)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran (*R*-THMF, red pentagons) is found bound to a homologue of the periplasmic binding protein LsrB. LsrB is part of an ABC transporter (encoded by the *lsrACDBFGE* operon). The putative ATPase of the ABC transporter (LsrA), sugar binding protein (LsrB), heterodimeric membrane channel (LsrC and LsrD), LsrF and LsrG show similarity to proteins encoded by the *b1513* operon in *Escherichia coli* [45], and similar operons exist elsewhere. This operon is regulated by the repressor LsrR, the gene of which is located upstream. Immediately downstream of *lsrR* is a gene (*lsrK*) with similarity to the xylulokinase gene (*xylB*). LsrK phosphorylates AI-2, probably following import to sequester it in the cytoplasm. Phosphorylated AI-2 then causes LsrR to relieve its repression of the *lsr* operon, allowing further AI-2 import. LsrF and LsrG are necessary for further processing of phospho-AI-2 and form a product(s) no longer capable of preventing the repression by LsrR. It is not clear whether LsrF (which resembles an aldolase), or LsrG (unknown function) act on different substrates (for example, phosphates at different positions of AI-2, or chemically distinct forms of AI-2), or whether they catalyse reversible reactions that can occur spontaneously at a low level. It is however clear that both are required for the further processing of phospho-AI-2. The product of the gene with similarity to ribulose phosphate epimerase (LsrE) is thought to contribute to this conversion, but its function is unclear[46]. The alternative pathway of AI-2 response described for *Vibrio* spp. involves a phosphorelay signalling transduction (shown on the right). The form of AI-2 that is active in this system is the furanosyl-borate-diester (*S*-THMF-borate: green double pentagons) in complex with the periplasmic binding protein, LuxP[33]. The current model suggests that LuxP then interacts with the inner membrane protein, LuxQ, inducing a conformational change that confers a phosphatase activity in LuxQ. This process extracts a phosphate from a two-component phosphorelay protein (LuxU) that in turn dephosphorylates the response regulator, LuxO[38–40], leading to activation of bioluminescence[38,119]. At low cell densities (in the absence of AI-2), the proteins involved are converted into kinases, which reverses the flow of phosphates to create phosphorylated LuxO[40]. Phosphorylated LuxO acts alongside σ^{54} to activate the production of multiple, redundant small regulatory RNAs (sRNAs) which interact with the mRNA of the transcriptional activator $LuxR_{Vh}$ (HapR in *Vibrio cholerae*), causing Hfq-dependent destabilization. As $LuxR_{Vh}$ activates transcription of the *luxCDABEGH* operon (which encodes the enzymes required for light generation), the reduced levels of $LuxR_{Vh}$ protein in the cells leads to a reduction in bioluminescence[42]. As the dephosphorylated LuxO generated by the presence of AI-2 (at high cell densities) can not activate the production of sRNAs, the $luxR_{Vh}$ mRNA has an increased stability, and the resultant $LuxR_{Vh}$ induces bioluminescence by activating the *luxCDABEGH* operon. The phosphorelay is depicted by the arrowed circles containing 'P', and the direction of transfer in the presence of AI-2 is indicated by the solid arrows. Figure based on data from REFS 42,46.

First, crystallographic analysis of the AI-2-binding protein of *V. harveyi*, LuxP (which clearly has a role in cell–cell communication), revealed that the protein was complexed to a FURANOSYL-BORATE-DIESTER[33] (3A-methyl-5,6-dihydro-furo(2,3-D)(1,3,2)dioxaborole-2,2,6,6A-tetraol; *S*-THMF-borate) (FIGS 1,2). This molecule was suggested to arise by cyclization of DPD followed by reaction with borate (FIG. 1). However, AI-2 activity is not always associated with boron, as the *Salmonella enterica* serovar Typhimurium (*S. typhimurium*) AI-2-binding protein LsrB (LuxS-regulated protein B) (FIG. 2), interacts with (*2R,4S*)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran (*R*-THMF), which lacks boron[34].

Despite low sequence identity between LsrB and LuxP, they have the same fold, although LsrB shows strong structural homology with several other sugar-binding proteins and repressors of the LacI/RsbB family[34]. Moreover, the AI-2 binding sites are distinct: the polar residues in LuxP are replaced with non-polar residues in LsrB, which generates a different net charge and orientation in the binding sites. Despite the differing nature of molecules interacting with AI-2-binding proteins, Miller *et al.*[34] demonstrated their capacity for interconversion, and the influence that boric acid has on the equilibrium between them[34]. It is probable that, depending on the niche of a bacterium, a chemically different form of AI-2 might be active (for example, in the marine environment where borate is plentiful, the predominant form of AI-2 is the furanosyl-borate-diester). Alternatively, the different molecular structures might reflect variation in the function of AI-2 (quorum sensing versus metabolic).

Schemes for the potential interconversions of all the molecules implicated with AI-2 activity are shown in FIG. 1. However, the alternative (non-LuxS-dependent) intracellular sources of DPD which can be generated from spontaneous degradation of sugar phosphates[35,36] are not displayed.

Functions of AI-2

In the light of the intriguing possibility that the different AI-2-binding proteins could participate in the mediating of distinctly different responses to AI-2, we now consider the two possible functions of AI-2 — as an autoinducer or as a metabolite — in the context of these responses.

***Response to AI-2 by* V. harveyi.** The clearest evidence that demonstrates that AI-2 can control gene expression through quorum sensing comes from studies of *Vibrio* spp., in particular the organism in which it was first identified, *V. harveyi.* AI-2 is one of three quorum-sensing signal molecules produced by *V. harveyi* that control bioluminescence (BOX 1). AI-2 can gain access to the periplasm of *V. harveyi*, where it interacts with the periplasmic binding protein LuxP, which in turn interacts with a membrane-bound histidine protein kinase, LuxQ[33,37]. In the absence of AI-2, LuxQ auto-phosphorylates[38–40] and transfers a phosphate to LuxU, which subsequently passes it to the response regulator,

Box 1 | Quorum-sensing signal molecules of *Vibrio* species

Vibrio **spp. strains can use multiple communication signals. *Vibrio harveyi* has a complex quorum-sensing system using signal molecules from three different families, whereas *Vibrio cholerae* uses representatives of two families. Both species use autoinducer-2 (AI-2) and a molecule first described in *V. cholerae*, cholerae autoinducer 1 (CAI-1). In addition to these, *V. harveyi* also produces and responds to HAI-1** (REFS 41,69)**.**

CAI-1 belongs to a new class of uncharacterized signal molecules. Preliminary studies indicate that CAI-1 production is not widespread, as it was only found in *Vibrio* spp. and closely related marine bacteria. Synthesis of CAI-1 depends on putative aminotransferase CqsA homologues, and its sensing uses two-component hybrid sensor kinase CqsS homologues[41,69].

HAI-1 is an *N*-acyl homoserine lactone (*N*-AHL). Although usually synthesized by proteins homologous to LuxI, *N*-AHLs can be generated by other enzymes such as HtdS, or, in *V. harveyi*, LuxM homologues. Generally, the specificity of these signal molecules is conferred by the length and oxidation state of the acyl side-chain attached to the homoserine lactone ring, and is achieved through their binding to and activation of a transcriptional regulator homologous to LuxR. Note that LuxR is distinct from $LuxR_{Vh}$, being related to the TetR family of transcriptional regulators, and functions independently of the signal molecule itself. In *V. harveyi*, sensing of HAI-1 does not involve a LuxR homologue, but instead the hybrid sensor kinase LuxN, which consists of both a sensor kinase domain and a response-regulator domain[114,115].

To integrate so many concomitant signals, *V. harveyi* needs an effective coordination system, which is provided by the phosphotransferase LuxU, as all three sensors, that is, LuxN (AI-1), LuxQ (AI-2), and CqsS (CAI-1), relay phosphate to LuxU at low signal concentrations. This could allow 'three-way coincidence detection' for the regulation of the expression of target genes. It is suggested that signal strength follows the kinase-to-phosphatase ratio of each receptor, LuxN>LuxQ>CqsS, but this might vary with the environmental conditions[41,43]. Two hypotheses currently explain such a level of signal complexity: by multiplying the signal molecules, the bacterium protects itself against noise caused by the presence of similar molecules of heterologous origin, and it allows the detection of the possible eight different population states.

LuxO. Phospho-LuxO, with σ^{54}, activates expression of small regulatory RNA (sRNA), which, with the help of the chaperone Hfq, destabilizes the mRNA that encodes the activator protein $LuxR_{Vh}$. As $LuxR_{Vh}$ is required for transcription of the luciferase-encoding genes, no light is generated. At high AI-2 concentrations (indicating high cell density), LuxQ functions as a phosphatase, reversing the flow of phosphates and turning on light production (FIG. 2). The sensors of the other two autoinducers (AI-1 and CAI-1 (cholerae AI-1), see BOX 1) feed into the same pathway at the level of LuxU. The components of the AI-2 response network are shared by other *Vibrio* species (including *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificus* and *Vibrio fischeri*, as well as the renamed *Listonella anguillarum*[41,42]).

This mechanism integrates the sensory inputs from all the autoinducers; however, *V. harveyi* can distinguish between conditions in which all autoinducers are present (the 'coincidence state', characterized by complete dephosphorylation of LuxO) and all other variations[41,43]. So, in *V. harveyi*, the individual presence of an autoinducer initiates a phosphatase activity that exceeds the remaining kinase activity, but is not sufficient to deplete phospho-LuxO completely. Therefore, the simultaneous presence of all three autoinducers will result in a more dramatic effect on gene regulation. The sensitivity of this system might even enable *V. harveyi* to respond to changes in the ratio between AI-1 and AI-2 (REF. 43). The involvement of sRNAs in this system might impart the ability to behave as a sensitive, definitive on/off switch leading to an all-or-nothing response[42]. Moreover, it is not dedicated to the control of bioluminescence, but can modulate other phenotypes in *V. harveyi*[43,44].

***Uptake of AI-2 by* S. typhimurium *and* E. coli.** In *S. typhimurium*[45,46] and *E. coli* (K.W. and K.R.H., unpublished results and REF. 47), instead of initiating a phosphorylation cascade, AI-2 seems to be taken up by an ABC TRANSPORTER. As observed with other bacteria, AI-2 is only transiently found in culture supernatants[19]. Although AI-2 might undergo inactivation or degradation in the supernatant or on the cell surface (either spontaneously or enzymatically), some bacterial cells can actively take up AI-2, and the genes encoding an ABC transporter were identified as an operon that is differentially expressed in a *S. typhimurium luxS* mutant (the *luxS*-regulated *lsr* operon)[45]. Immediately upstream of this *lsrACDBFGE* operon are the divergently transcribed *lsrR* gene (encoding a repressor for the *lsr* operon), and *lsrK* (encoding a kinase responsible for AI-2 phosphorylation). Deletion of *lsrR* results in rapid and complete removal of AI-2 from the supernatant, presumably because the *lsr* operon is maximally expressed and the encoded proteins transport all the AI-2 into the cell[45]. The combined effect of the ABC transporter and adjacent genes is to phosphorylate AI-2 and then further convert it to currently uncharacterized product(s) (FIG. 2). Recently, the *E. coli* genes homologous to *lsr* have been shown to have similar functions[47].

As *lsr* transporter mutants retain the ability to slowly internalize AI-2, it appears that there is a second, inefficient AI-2 transporter. Given the structure of AI-2 and the similarity of the *lsr* operon with ribose-uptake systems (rbs), it is possible that the latter could function in this regard. However, the *rbs* operon of *E. coli* does not seem to have this function[48]. An alternative transporter normally used to transport other molecules with a similar structure to AI-2 (including MHF, see BOX 2) could perform this role (unpublished data, see below). Although also described as a quorum-sensing-related response[46], the similarity (both by sequence homology and function, that is, uptake followed by phosphorylation) of the Lsr transporter with the uptake mechanisms of other sugars strongly indicates a primarily metabolic role for AI-2.

Linkage of *luxS* to methionine synthesis genes

It has been suggested that the role of *luxS* is unlikely to be metabolic, as *pfs* is present in some bacteria even though *luxS* is not, which indicates that Pfs itself is sufficient for detoxification of SAH[18] (see Supplementary information S1 (figure)). The ease of construction of *luxS* mutants also argues against an essential role, as does the lack of growth defects of a *S. typhimurium luxS* mutant, and its inability to grow in minimal media containing AI-2 as the sole carbon source[46]. However, a long-term selective advantage is probably conferred by the ability to recycle metabolic building blocks.

S-RIBOSYLHOMOCYSTEINE
Substrate of LuxS, product of action of Pfs on *S*-adenosyl-L-homocysteine.

4,5,-DIHYDROXY-2,3-PENTANEDIONE
Product of LuxS reaction.

HOMOCYSTEINE
Sulphur-containing precursor of methionine in activated methyl cycle.

4-HYDROXY-5-METHYL-3(2H)-FURANONE
Volatile, naturally occurring furanone derivative.

FURANOSYL-BORATE-DIESTER
Molecule bound to LuxP.

R-THMF
Molecule bound to LsrB.

σ^{54}
Alternative sigma (σ) transcription factor, also called RpoN, which promotes the RNA core-enzyme activity in response to nitrogen and carbon environmental signals.

Box 2 | **Evolution of signalling: insights from autoinducer-2 (AI-2) response networks**

One possible mechanism underlying the evolution of cell–cell signalling might be through the exploitation of cell-excluded metabolites by bacteria. The *Pseudomonas aeruginosa* siderophore pyoverdine, for instance, functions as a signal molecule in addition to scavenging iron[116]. The growing family of 4,5,-DIHYDROXY-2,3-PENTANEDIONE (DPD)-derived furanone derivatives might represent yet another example.

Bacteria can express *luxS* and produce AI-2 in conjunction with a phosphorelay signal transduction pathway that incorporates a LuxP homologue, which indicates a quorum-sensing role such as found in *Vibrio* spp. Alternatively, an ABC transporter and an AI-2-phosphorylating kinase might be present (for example, *Salmonella typhimurium lsr* system). The latter system resembles those involved in the use of certain pentose sugars and implies a metabolic function for AI-2. Interestingly, there are also examples of bacteria containing a complete Lsr transporter/kinase system in the absence of LuxS, for example, *Sinorhizobium meliloti*. Is it possible that these systems are used for true interspecies communication, or are they scavenging for a valuable nutrient?

The nine conserved residues of the LuxP ligand-binding site can be used to search for proteins with a similar function, and it is intriguing that their genes are located in three different contexts. First, they can be linked to genes encoding parts of a typical signal transduction cascade (for example, *Vibrio* spp.). Second, as orphan genes (for example, δ-proteobacterium *Desulfovibrio desulfuricans* G20), and last, genetically linked to an ABC transporter (for example, the marine α-proteobacterium *Silicibacter pomeroyi* DSS-3). Intriguingly, *D. desulfuricans* and *S. pomeroyi* do not contain a *luxS* gene but use the SAH (*S*-adenosyl-L-homocysteine) hydrolase pathway to degrade SAH. Are the putative LuxP homologues in these organisms used for signalling, or to acquire a metabolite? Do the ligands in these cases contain boron, given their link with marine bacteria? Although not all the species listed above always inhabit a marine environment, they might contain an AI-2-responsive transduction system comprising in part a LuxP homologue, because of their close relationship to marine species and evolutionary origin. By contrast, the *lsr* system seems to be present in terrestrial bacteria only. Do other AI-2 detection systems exist? One possibility is a transporter also capable of transporting 4-hydroxy-5-methyl-3(2H)-furanone (MHF) (unpublished data, see below).

In support of a metabolic role, several organisms have been isolated that can use the DPD derivative MHF as their sole source of carbon[117]. Therefore, it is tempting to speculate that DPD-derived furanones, some of which are toxic[118], originally represented a class of (transiently) excreted metabolites and, in the marine environment, furanosyl-borate-diester (as the predominant form of these metabolites) might have been adopted as a signal molecule. DPD-derived furanones are particularly suited for this role, as their production is directly linked with the metabolic flux through the activated methyl cycle[10,] (theoretically, 1 molecule of DPD is formed per *S*-adenosyl-L-methionine-dependent methylation reaction) making their concentration, under ideal conditions, an accurate measure of cell number[18] or metabolic activity[82].

Interestingly, *luxS* and *pfs* have been found adjacent to other genes involved in metabolic reactions linked to the AMC, in particular those involved in the metabolism of sulphur-containing amino acids, such as *cysK*, *metB*, and *metE/H*, which are required for cysteine and methionine biosynthesis respectively (REF. 19) (FIG. 1).

There is speculation about the possible evolutionary steps that have resulted in the genetic linkages described above. Hypotheses to explain the possession of one or more of the alternative MET recycling routes are also developing — why do some bacteria harbour *luxS* and *pfs* genes together, whereas others bear the gene encoding SahH (the enzyme capable of converting SAH to homocysteine without concomitant production of DPD)? Lerat and Moran[49] examined the phylogeny of *luxS* homologues and, based on the close similarity of their *luxS* genes, concluded that horizontal transfer had probably occurred from the Firmicutes to distantly related bacteria, such as the ε-proteobacterium *Helicobacter pylori*, the actinobacterium *Bifidobacterium longum*, and the spirochaete *Borrelia burgdorferi*. Generally, within genera or higher order groups, there is conservation of one particular pathway (see Supplementary information S1 (figure)), and the first impression is that many pathogenic species possess LuxS, whereas many non-pathogenic species harbour SahH. However, given the current bias in sequenced strains it is too early to generalize.

ABC TRANSPORTER
Transporter protein containing an ATP-binding cassette.

The roles of LuxS/AI-2 in pathogenesis

With such a wide distribution, it is not surprising that the LuxS protein has an important role in infection, and in support of this assertion, experimental studies (which tend to focus on the mechanisms that underlie virulence) have highlighted a role for LuxS in pathogenesis. The remainder of this review focuses on our understanding of the LuxS-dependent phenotypes of pathogens that inhabit certain niches within the host.

With LuxS and AI-2 having two potential roles (metabolic and quorum sensing), disruption of either could cause the observed phenotypic changes. Efforts have been made to highlight what might be the more plausible situation in each bacterium; however, it is clear that more careful analysis is required to precisely delineate the mechanism behind the phenotypic alterations. Additionally, many bacteria harbour complex regulatory circuits, as illustrated by the enterics[50–53,] which can be indirectly influenced through altered metabolism. Therefore, this article has focused on regulatory aspects relating to AI-2 and AHL signalling only.

Oral pathogens

The oral cavity contains many bacteria, including over 300 different species that colonize the three principal surfaces available in the oropharynx[54]. Teeth and mucosal epithelial cells are populated by micro-organisms that form plaque, which itself provides a

further niche for colonization, leading to mixed species communities. These polymicrobial aggregations are crucial for the pathogenesis of oral disease. Gram-positive (for example, *Actinomyces* spp.) and Gram-negative (for example, *Fusobacterium* spp.) bacteria together promote biofilm and dental plaque formation, which lead to inflammation of gums (gingivitis) and tooth decay[54]. Therefore, the oral cavity is an ideal ecological niche where bacteria could interact and communicate with each other. Previous studies have shown that peptides secreted by staphylococci can change the behaviour of enterococci, acting like endogenously produced pheromones[55]. However, a shared AI-2 quorum-sensing system has the potential to offer a broader scope for communication between bacterial species.

Several oral pathogens produce AI-2. A survey of 33 strains (including 16 different species) from the oral cavity found that about half could induce bioluminescence of the *V. harveyi* biosensor[56]; however, these strains represented only three genera, *Fusobacterium, Prevotella* and *Porphyromonas*[56]. Additional evidence for widespread AI-2 production comes from the identification of *luxS* homologues among various oral pathogens, including *Prevotella intermedia* strains, *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans*, although it is intriguing that the sequenced *Fusobacterium nucleatum* strains apparently lack a *luxS* homologue (see Supplementary information S1 (figure)).

A *P. gingivalis luxS* mutant showed reduced expression of a TonB homologue, an excinuclease and a haemin-regulated receptor[57]. TonB is a component of a periplasmic complex that is responsible for the transport of iron from outer-membrane receptors into the cytoplasm. LuxS also influenced production of two extracellular cysteine proteases and a haemagglutinin[58]. Despite these reductions in expression of potential virulence determinants, the *P. gingivalis luxS* mutant retained its virulence in mice after subcutaneous inoculation[58]. However, although the *P. gingivalis luxS* mutant retained its ability to form biofilms with wild-type *Streptococcus gordonii*[57], it failed to do so with a *luxS* mutant of *S. gordonii*[59].

Several genes in *A. actinomycetemcomitans* are regulated in an AI-2-dependent fashion[60], including *afuA* (*A. actinomycetemcomitans* ferric uptake protein A, which encodes a periplasmic protein involved in iron transport), and a gene encoding leukotoxin (a member of the RTX (repeats in toxin) family of toxins). The reduced expression of AfuA has important functional consequences, as strains lacking *luxS* have a growth defect in iron-restricted conditions which is probably crucial *in vivo*[61]. The regulation of genes involved in iron metabolism in both *A. actinomycetemcomitans* and *P. gingivalis* suggests that AI-2 might function as a signal to bacteria that indicates nutrient limitation[57,61].

Two of the main components of dental plaque biofilm, *S. gordonii* and *Streptococcus mutans*, harbour a *luxS* homologue[59,62]. Disruption of either of these *luxS* homologues leads to strains that form biofilms with altered architecture[62,63]. As *S. gordonii* is an initial colonizer of freshly cleaned enamel, the alteration in biofilm formation over the 4-hour period studied might adversely affect its ability to initiate infection. The aggregates formed by LuxS-negative *S. mutans* were more resistant to antibiotics and detergents than the wild type, which indicates that the virulence of this microorganism could also be altered. This suggestion is supported by gene expression changes in the *luxS* mutant of *S. mutans*, including downregulation of the virulence determinant fructanase by more than 50% (REF. 64). It is probable that many other bacteria in the oropharynx produce AI-2-like molecules, and further understanding of the complex interactions between organisms will require more work using mixtures of bacterial species.

Gastrointestinal pathogens

Helicobacter pylori. *H. pylori* is the leading cause of peptic ulceration and inhabits the mucous layer that lines the gastric lumen. Initially, no phenotype, not even proteomic variations, could be attributed to a *H. pylori luxS* mutant, apart from the defect in AI-2 production[65,66]. More recently, it was demonstrated that a *luxS* mutant of *H. pylori* formed biofilms more efficiently than the parent strain[67], and no longer displayed growth-phase-dependent expression of one of the flagellin-encoding genes, *flaA*. However, it is not known whether this particular phenotype affects motility of the mutant strain[68].

Several enteric pathogens inhabit the lower gastrointestinal tract and cause localized disease following their acquisition through the faecal–oral route. As hundreds of different bacterial species are present in the intestine, there are ample opportunities for the microbial community to interact with each other. Many enteric pathogens produce and respond to AI-2, but whereas the biosynthetic pathway of AI-2 formation is identical in *E. coli*, *S. typhimurium* and *V. cholerae*[32,34,69], the genes and phenotypes regulated by the LuxS/AI-2 system are distinct.

***Enteropathogenic and enterohaemorrhagic* E. coli.** Strains of pathogenic *E. coli* colonize the intestine, and several produce potent enterotoxins that cause life-threatening diarrhoeal disease or urinary tract infections[70]. Enteropathogenic *E. coli* (EPEC) colonizes the mucosa of the small intestine and causes diarrhoea in infants and small children, without invading host cells or producing toxins. On the other hand, enterohaemorrhagic *E. coli* (EHEC) colonizes the large intestine and secretes a Shiga-like toxin, which is largely responsible for haemorrhagic colitis and haemolytic uraemic syndrome that sometimes occur following infection[70,71]. Both EPEC and EHEC subvert intestinal epithelial cell function to produce a characteristic histopathological feature known as the 'attaching and effacing' (A/E) lesion[70].

The genes required for formation of A/E lesions by EHEC and EPEC are encoded in a pathogenicity island called the locus of enterocyte effacement (LEE). In EPEC, the LEE contains 41 genes, which encode: a

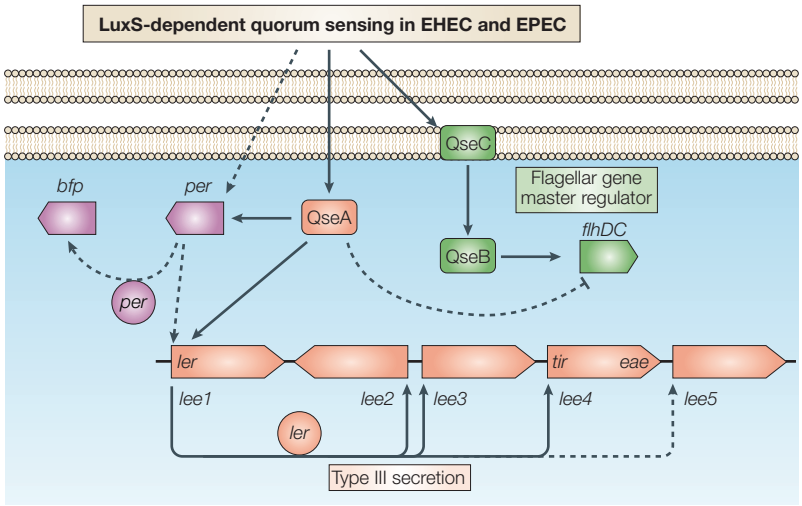

Figure 3 | **Model for LuxS-dependent quorum sensing in enterohaemorrhagic (EHEC) and enteropathogenic (EPEC) *Escherichia coli*.** Interactions limited to EPEC are indicated by dashed lines. The histidine kinase QseC (quorum-sensing *E. coli* regulator C), together with its cognate response regulator QseB, activates the flagellar master operon *flhDC* (green boxes). Regulation of type III secretion (red boxes) is mediated through QseA (a LysR homologue) and, indirectly, through Ler. Together, the two proteins activate the expression of the five important *lee* operons, encoding components of the type III secretion apparatus as well as an adhesin (intimin, Eae), the intimin adhesin receptor Tir, and other proteins. Transcription of *ler* (LEE-encoded regulator) (the first gene in the *lee1* operon) is activated by QseA. In EPEC, the plasmid-encoded AraC homologue Per is also thought to mediate quorum-sensing (QseA)-dependent regulation of *ler* and *bfp* (bundle forming pilus). Not shown is the postulated quorum-sensing-dependent activity of yet unidentified transcription factors in both EHEC and EPEC, and the auto regulation (repression) of QseA in the latter. Figure based on data from REFS 73,76.

TYPE III SECRETION SYSTEM (TTSS), which is an important mechanism for delivery of bacterial proteins directly into host cells; proteins secreted by the TTSS, which are essential for signal transduction in host cells and to A/E lesion formation; and the adhesin intimin together with its receptor, Tir[70,72].

The transcriptional regulation of genes in the LEE is complex (FIG. 3). Two transcriptional regulators (QseA (quorum-sensing *E.coli* regulator A), a LysR-like regulator[73], and the LEE-encoded regulator, Ler) participate in LEE operon regulation in EPEC and EHEC, with EPEC having an additional influence through Per (plasmid-encoded regulator). Ler is encoded by the *lee1* operon and influences expression of LEE2, LEE3 and *tir* operons in EHEC. In EPEC, Ler also modulates LEE4 expression[74]. Quorum sensing was initially proposed to directly affect LEE1 and LEE2 expression in EPEC, and to indirectly influence expression of other LEE operons through Ler[75]. Recently, this model has been refined, and *luxS*-mediated quorum sensing is now suggested to function through QseA, and possibly other factors[73]. In EPEC, *luxS* and *qseA* mutants show decreased adhesion to epithelial cells, decreased TTSS gene expression, and display either increased (*qseA* mutant) or decreased (*luxS* mutant) flagellin production and motility[76]. In EHEC, mutation of *luxS* altered flagellation and motility. However, although QseA influenced TTSS, it had no effect on the flagellar regulon[73]. Instead, it has been suggested that a novel two-component system (QseBC) regulates flagellar biogenesis and motility in EHEC[77]. It seems that QseBC is inhibited by QseA in EPEC, and that quorum sensing can also function through Per[76].

Why do EHEC and EPEC show differences in the regulation by quorum sensing? Given that multiple bacterial species reside in the intestine and that EHEC has a remarkably low infectious dose (around 10 bacteria), intestinal colonization could be induced by quorum-sensing signals produced by the normal intestinal flora[75]. As EPEC colonizes the proximal small intestine, where there are fewer resident bacteria, Per might provide compensation for the lack of exogenous signals, leading to the suggestion that EPEC uses quorum sensing for intraspecies signalling, whereas EHEC exploits AI-2 for interspecies signalling. The difference between flagella regulation of EHEC and EPEC through QseA might reflect different roles of flagella in EPEC and EHEC during pathogenesis. In EPEC, flagella have an involvement in adherence and microcolony formation not shared by EHEC strains[76].

As preliminary microarray analysis indicated that around 10% of all genes are differentially expressed in a *luxS* mutant of EHEC[78], it was suggested that quorum sensing was a global regulatory mechanism for basic physiological functions of *E. coli* as well as for the production of virulence factors. However, despite a reliance on LuxS function, *in vitro*-synthesized AI-2 did not modulate the TTSS of EHEC[79]. Instead, it was proposed that another molecule (AI-3) could be produced by LuxS. AI-3 remains chemically uncharacterized, and is proposed to interface with the host adrenaline signalling system[79], so AI-3 might be responsible for the effects described above instead of AI-2. However, as the nature of AI-3 remains unknown, its effects have not been rigorously investigated, and it remains to be proven whether AI-3 production is linked to the presence of LuxS in other bacteria, and if it is a direct product of the synthetic reaction catalysed by LuxS instead of a consequence of physiological changes associated with the AMC[80].

Salmonella. Over 1,400 serotypes of various *Salmonella* species can cause foodborne gastroenteritis, resulting from colonization of the small and large intestine, and marked acute inflammatory responses in the gastrointestinal tract[81]. In *S. typhimurium*, AI-2 production is tightly correlated with *pfs* transcription[82]. This could be explained by an increased need for SAH detoxification, leading to raised levels of Pfs, which in turn result in elevated SRH levels and LuxS activity. This generates higher levels of AI-2 and indicates a metabolic function instead of a quorum-sensing-related function. In support of this, the only genes currently known to be regulated by AI-2 in *S. typhimurium* are the seven-gene *lsr* operon, which promotes internalization of AI-2 (REFS 45,46,83). This finding is consistent with the Lsr ABC transporter acting to take up AI-2 and its use as a metabolite, and indicates that metabolic changes in *luxS* mutants can

TYPE III SECRETION SYSTEM
A syringe-like proteinaceous machinery that can transport bacterial proteins directly into a eukaryotic cell by injection.

Table 1a | **Phenotypic changes affected by LuxS or autoinducer-2 (AI-2) in pathogenic bacteria**

Bacterium	Phenotype	Complementation with *luxS* gene	Complementation with *in vitro* synthesized AI-2	Complementation with CM containing AI-2	Refs
Actinobacillus actinomycetemcomitans	Increased leukotoxin and *afuA* expression*	N/A	ND	N/A	60
Borellia burgdorferi	Proteome modulation*	N/A	ND	N/A	103
Campylobacter jejuni	Decreased motility	ND	ND	ND	86,87
	Reduced autoagglutination	ND	ND	ND	87
Clostridium perfringens	Reduced toxin production	ND	ND	Full	98
	Reduced *pfoA* mRNA	Partial	ND	Full	98
EHEC	Upregulation of *lee* operons‡	N/A	ND	N/A	75
		N/A	None Attributed to AI-3	N/A	79
	Expression of 404 genes altered (single microarray)	ND	ND	ND	78
	Shorter generation time	Full	ND	Full	78
	Motility	Partial (multicopy *luxS*, inconsistencies)	ND	Partial	78
	Activation of *qseA* transcription	Partial	ND	Full	73
	Downregulation *qseBC* transcript	Full	ND	Full	77
	Decreased type III secretion	Full	None	ND	79
EPEC	Upregulation of *lee* operons‡	N/A	ND	N/A	75
	Reduced secreted proteins	Full	ND	ND	75
	Reduced per expression	Full	ND	ND	76
	Type III secretion reduced	Full	ND	ND	76
	Adhesion to HeLa cells	Full	ND	ND	76
	Motility reduced	Full	ND	ND	76
Helicobacter pylori	Increased biofilm	ND	ND	ND	67
	flaA expression reduced	Full	ND	Partial	68
Neisseria meningitidis	Attenuation *in vivo*	Full	ND	ND	91
	Siderophore receptor transcript increased (microarray compared to WT)	ND	ND	ND	94
	MetE and MetF increased (proteome comparing +/– *in vitro* synthesized AI-2)	ND	Attributed to homocysteine	ND	95
Porphyromonas gingivalis	Reduced TonB, excinuclease, hemin-regulated receptor, increased hemin acquisition protein, Rgp	ND	ND	ND	57
	Reduced Rgp & Kgp proteases, haemagglutinin levels	ND	ND	ND	58
	Failed to form biofilm with *S. gordonii luxS* mutant	ND	ND	Formed biofilm with WT *S. gordonii*	59
	Increased *hasF,* decreased *uvrB* expression	ND	ND	Full. CM from *E. coli* containing *luxS* from *A. actinomycetemcomitans.* Comparison to sterile broth	60

For each phenotype it is noted whether complementation of a *luxS* mutant was attempted genetically (with a *luxS* gene), or through addition of AI-2 in CM or synthesized *in vitro*. CM is obtained from cell-free supernatant of a *luxS*$^+$ source (AI-2$^+$) and the presence of AI-2 activity verified by using a *Vibrio harveyi* assay. In each case the negative control was CM harvested from a *luxS* mutant (lacking AI-2) unless otherwise indicated. Note that only one study successfully used synthesized AI-2 for complementation of a *luxS* mutant. On the only other two occasions when this was attempted, phenotypic modulations were attributed to another molecule (either homocysteine or AI-3). Complementation was either full or partial, as indicated. This also applies to TABLE 2b. *Phenotype of wild-type strain modulated by CM containing AI-2 in comparison to non AI-2-containing CM. ‡Phenotype of gene fusion in *E. coli* K12 modulated by CM containing AI-2 from WT in comparison to non AI-2-containing CM. AI-2, autoinducer-2; CM, conditioned medium; EHEC, enterohaemorrhagic *Escherichia coli*; EPEC, enteropathogenic *Escherichia coli*; ND, not determined; N/A, not applicable; WT, wild type.

have consequences in gene regulation. For example, the genetic screen used by Taga *et al.*[45] identified *metE*, which encodes an enzyme integral to *de novo* methionine synthesis, as a gene with altered expression in a *luxS* mutant when compared with the parental strain. This indicates that the AMC influences metabolite levels and therefore gene expression, and provides an illustration of how *luxS* phenotypes can be misinterpreted. There are no other descriptions of AI-2-mediated signalling in *Salmonella*.

Table 1b | **Phenotypic changes affected by LuxS or autoinducer-2 (AI-2) in pathogenic bacteria**

Bacterium	Phenotype	Complementation with *luxS* gene	Complementation with *in vitro* synthesized AI-2	Complementation with CM containing AI-2	Refs
Salmonella enterica	Increased *lsr* operon transcript	Full	Full, compared to synthesis buffer	ND	45
	Increased *metE* transcript	Full	None	ND	45
Serratia marcescens strain 39006	Decreased carbapenem, normal prodigiosin and virulence	ND	ND	ND	107
Serratia marcescens strain 274	Decreased prodigiosin, haemolysin	Full (*S. marcescens* and *E. coli luxS*)	ND	Full	107
	Decreased virulence in *Caenorhabditis elegans* model	ND	ND	ND	107
Serratia marcescens strain 12	Increase in mucoidy	ND	ND	ND	107
Shigella flexneri	*virB* expression decreased	ND	ND	Partial Compared to LB	90
Streptococcus gordonii	Altered biofilm morphology	ND	ND	ND	63
	Failed to form biofilm with *Porphyromonas gingivalis luxS* mutant	Full	ND	ND	59
	Decrease *gtfG*, *fruA*, *lacD*	ND	ND	ND	59
Streptococcus mutans	Altered biofilm	ND	ND	ND	62,64
	Decreased *fruA* expression	ND	ND	Full	64
	Increased acid sensitivity	ND	ND	Full	64
	Downregulation of *ffh*, *brpA*, *recA*, *smnA*, *nth*	ND	ND	ND	64
Streptococcus pneumoniae	Altered transcriptional profiles	ND	ND	None	93
	Attenuated in mouse	ND	ND	ND	93
	Altered proteome	ND	ND	ND	92
Streptococcus pyogenes	Increased streptolysin S	ND	ND	ND	96
	Increased *sagA* expression	ND	ND	ND	96
	Decreased growth rate (media dependent)	ND	ND	ND	96
	Decreased SpeB (post-transcriptional effect)	ND	ND	ND	96
	Increased Hep-2 cell internalisation	ND	ND	ND	97
	Increased *emm3* expression	ND	ND	ND	97
	Decreased *speB* expression	ND	ND	ND	97
	Reduced HA capsule	ND	ND	ND	97
Vibrio cholerae	*tcp* expression modulated*	N/A	N/A	N/A	69
Vibrio vulnificus	Haemolysin increased	Partial	ND	Partial	100
	Delayed/decreased protease production	Full	ND	Full	100
	Reduced virulence *in vivo*	ND	ND	ND	100
	Decreased cytotoxicity to macrophages‡ and HeLa cells	ND	ND	ND	100, 101

*Phenotype of CAI-1$^-$, AI-2$^-$ (HapR+) strain modulated by CM from CAI-1$^-$ in comparison to CM from AI-2$^-$ strain. Other modulations attributed to quorum-sensing regulation in *V. cholerae* address AI-2 signalling indirectly by analysing mutants in the response cascade[26,84]. ‡Double *smcR luxS* mutant compared to wild type. CM, conditioned medium; ND, not determined; N/A, not applicable.

Vibrio cholerae. Intestinal infection with *V. cholerae* results in the severe diarrhoeal disease cholera through the action of the cholera toxin, a potent stimulator of adenylate cyclase. *V. cholerae* contains a complex quorum-sensing system similar to that of *V. harveyi*, which is used to regulate a wide range of phenotypes. Indeed, to some extent, the *Vibrio* spp. systems are interchangeable[42]. Much of the phenotypic characterization has centred around comparison of parental strains with mutants in the response cascade (including LuxU, LuxO and HapR) that follows both AI-2 and CAI-1 (BOX 1) stimulation. Analysis of these mutant strains revealed phenotypic alterations in the transcriptional regulator AphA (activator for *tcpP* and *tcpH* expression protein A), biofilm formation[84], cholera toxin, the toxin co-regulated pilus (TCP), haem-agglutinin protease, colonization of infant mice, and perhaps other virulence factors (although the latter requires verification of preliminary microarray data)[26,69]. Direct evidence for virulence-factor regulation by AI-2 has also been presented[69], and as people infected with *V. cholerae* produce antibodies against the AI-2 binding protein LuxP, signalling might be active *in vivo*[85].

Campylobacter jejuni. *C. jejuni*, a common cause of enteritis, also produces AI-2 activity. The *luxS* mutant is unaltered in its growth characteristics, invasiveness of Caco2 cells and resistance to oxidative stress induced

by paraquat and H_2O_2. However, there seems to be a reduction in the agglutination properties and the motility of a *luxS* mutant, although no controls were used to rule out phase-variable modulation of motility in the *luxS* mutants[86–88].

Shigella flexneri. The invasive enteric pathogen *Shigella flexneri* is a primary agent of bacillary dysentery (an acute inflammatory disease of human colonic epithelia that results from focal invasion and subsequent radial dissemination). The *S. flexneri* virulence plasmid contains the *ipa* (invasion plasmid antigens), *mxi* (membrane expression of invasion plasmid antigens) and *spa* (surface presentation of Ipa antigen) operons, which encode exported factors and a TTSS, as well as the transcription factors VirF (virulence transcriptional activator) and VirB[89]. The ability of culture supernatants containing AI-2 to mildly increase *virB* expression was interpreted as quorum-sensing modulation. However, although VirB is essential for expression of *ipa*, the culture supernatants had no influence on this, and *luxS* was not required for *S. flexneri* virulence. Possible explanations for this are that maximal expression of *virB* is not required for virulence, and that there are AI-2 responsive elements epistatic to *virB*[90].

Bacteraemia and meningitis

Neisseria meningitidis and *Streptococcus pneumoniae* are both important causes of bacteraemia and meningitis. These organisms are usually found colonizing the upper airway, and occasionally result in disseminated, bloodstream infection. In most individuals, spread to the central nervous system occurs through the bloodstream; *S. pneumoniae* can also cause fatal pneumonia. Both these bacteria have AI-2 activity that is dependent on *luxS* homologues, which are required for full virulence during disseminated infection[91–93]. A *S. pneumoniae* strain that lacks *luxS* could establish successful colonization of the murine nasopharynx, but did not spread to the lungs or cause bloodstream infection. The latter was probably due to an inability to replicate within the bloodstream instead of a failure to translocate from the nasopharynx, as the *luxS* mutant was also attenuated after intraperitoneal challenge; within 48 hours after challenge the *luxS* mutant could not be recovered in the spleen or bloodstream, whereas the level of wild-type bacteria was at least five orders of magnitude higher. In *S. pneumoniae,* disruption of *luxS* has pleiotropic effects on gene expression *in vitro*[93], reflected by detectable changes in at least ten uncharacterized proteins[92]. It is not known if these or any other changes are responsible for the attenuation.

The attenuation of a *N. meningitidis luxS* mutant was far less dramatic than for *S. pneumoniae*, as the *luxS* mutant was recovered at 20% of the level of the parental strain in the bloodstream following intraperitoneal administration[91]. This relatively minor attenuation might reflect the short time (22 hours) after infection when the bacteria were recovered. The model of meningococcal bacteraemia is abrupt, with maximal bacteraemia at 22–24 hours, after which animals either succumb or recover. There is no reliable animal model that faithfully reflects nasopharyngeal colonization by *N. meningitidis,* although a *luxS* mutant was found to be fully capable of adhering to human epithelial cells[94]. Microarray analysis of the transcriptional profile of the *N. meningitidis luxS* mutant was remarkably similar to that of the wild type when grown in the laboratory[94]. Independent proteomic analysis of a *N. meningitidis luxS* mutant grown both in the presence and absence of AI-2 also failed to demonstrate a global response[95]. It is therefore probable that, for *N. meningitidis* at least, the principle function of *luxS* is not the production of a signalling molecule but rather metabolism. If so, it is possible that the *luxS* mutant might have a modified transcriptome or proteome when grown in nutritional conditions that require *luxS* function.

Cutaneous and soft tissue infections

Streptococcus pyogenes. In *Streptococcus pyogenes*, which causes acute pharyngitis and infections of the skin and soft tissue (ranging from impetigo through to necrotizing fasciitis), LuxS contributes to the control of virulence gene expression[96]. A secreted cysteine protease, SpeB, and the haemolysin streptolysin are produced by the bacterium in a population-density-dependent fashion, and are each required for pathogenesis of skin infections. In a *S. pyogenes luxS* mutant, levels of SpeB are reduced, whereas those of streptolysin S are increased, which indicates the presence of two or more distinct regulatory pathways[96]. Whether the effect upon *speB* is manifest at the transcriptional or post-transcriptional level is unclear[96,97]. Several other phenotypes have also been detected in a *luxS* mutant, including an enhanced ability to enter epithelial cells[97] and a media-dependent growth defect[96]. The mechanism(s) for these phenotypes remains unexplained, in part because of the contradictory findings of the two published studies[96,97]. It is possible that the observed differences resulted from strain-specific effects or variant growth conditions.

Clostridium perfringens. *Clostridium perfringens* is an anaerobic Gram-positive bacterium that causes gas gangrene. The pathogenesis of infection is mediated by the production of multiple extracellular toxins and extracellular proteases, and *luxS* is co-transcribed with the metabolically linked genes *metB* and *cysK* (FIG. 1). Transcription of genes that encode three toxins, α-toxin, θ-toxin and κ-toxin, is reduced in a *luxS* mutant[98]. These toxins are produced at mid-exponential phase, although the initial transcription of the θ-toxin gene occurs before AI-2 is detectable, which indicates that a distinct mechanism governs early transcription of this gene. The production of toxins is restored in a *luxS* mutant by exposing the bacterium to culture supernatants from either *E. coli* harbouring a functional copy of $luxS_{Cp}$ or wild-type *C. perfringens* but not the corresponding *luxS* mutant[98]. The toxins are controlled by the two-component regulator, VirR/VirS[99],

and examining the effect of loss of *luxS* in a VirR/VirS-negative background indicated that the Vir system is required for LuxS-mediated regulation. The *in vivo* importance of these findings has not been assessed.

Vibrio vulnificus. *V. vulnificus* is closely related to *V. harveyi*, in which AI-2 was originally characterized, and contains a similar regulatory cascade[100]. The bacterium can cause necrotizing wound infections and fatal septicaemia in individuals exposed to contaminated fresh or salt water. Whereas protease production was delayed, there was increased haemolysin production by the *luxS* mutant, and both were at least partially restored by complementation[100]. Furthermore, the mutant was less cytotoxic (measured by LDH (lactate dehydrogenase) release) to human epithelial cells than the wild type, and the LD_{50} of the mutant was markedly reduced in murine models following intraperitoneal challenge[100,101].

Other pathogens

Proteus mirabilis. *Proteus mirabilis* is a motile Gram-negative bacterium that causes ascending urinary tract infection. Production of AI-2 by the bacterium coincides with initiation of swarming migration of colonies on agar plates, and the activity is maximal at the leading edge of the motile bacteria. However, despite careful phenotypic analysis of a *luxS* mutant, no biological function could be attributed to *luxS* or AI-2 production; the mutant showed normal swarming, colonial morphology and production of virulence factors such as proteases and urease. Furthermore, no attenuation could be identified in the murine model, either during colonization of the bladder or ascending infection of the kidneys[102].

Borrelia burgdorferi. *B. burgdorferi* is a spirochaete that causes Lyme disease, a chronic relapsing multisystem disease. In nature, the bacterium is maintained by cycles of infection between warm-blooded vertebrates and *Ixodes* spp. ticks, and is acquired by humans when an infected tick feeds on an individual. *B. burgdorferi* possesses a *luxS* gene which can functionally complement a *luxS*-deficient strain of *E. coli*, making it probable that *B. burgdorferi* produces AI-2 activity[103], though this has yet to be confirmed. The addition of conditioned media from the *luxS*-complemented *E. coli* strain to *Borrelia burgdorferi* cultures leads to marked changes in protein profiles. Some of these proteins have been identified, and include several outer membrane lipoproteins that bind Factor H, the inhibitor of the complement pathway, and could therefore affect resistance against innate immune killing in mammalian hosts[104]. LuxS does not contribute to the virulence of *B. burgdorferi* in a murine model following intradermal inoculation through tick or needle[105,106], which indicates that AI-2 signalling has no function in the mammalian host. However, *B. burgdorferi* might still require LuxS during its natural infectious cycle to regulate protein synthesis, potentially enabling a population of bacteria to coordinate the expression of virulence determinants during its lifecycle in ticks and/or transmission to the mammalian host.

Serratia marcescens. *Serratia marcescens* is an opportunistic human enteric pathogen that is responsible for many nosocomial infections and also causes disease in plants and invertebrates. Comparison of phenotypes revealed that mutation of *luxS* leads to decreased carbapenem antibiotic production in one *S. marcescens* strain, whereas it reduced levels of the antibiotic prodigiosin, haemolysin and virulence in a *Caenorhabditis elegans* model in another strain. The prodigiosin production could be complemented by the addition of spent culture supernatants; however, as discussed by the authors[107], this observation is not conclusive proof for a signalling role of AI-2 in this situation. This study is the most convincing evidence of strain-dependent effects of *luxS* mutation[107].

Concluding remarks

Many pathogens produce the LuxS protein and can produce AI-2, which, in some bacteria, regulates control over important virulence factors. Other microorganisms might use LuxS to complete important metabolic pathways linked either to methionine synthesis or furanone production. In the latter case, disruption of *luxS* could also result in decreased virulence owing to perturbations in general fitness. Therefore, whatever the primary function of LuxS is in a bacterium, it can contribute at several levels to the pathogenesis of infection, and further studies are required to elucidate these (TABLES 1a,b). One fascinating aspect is the strain-specific manifestation of some effects (for example, in *S. marcescens*, *S. pyogenes* and *C. jejuni*) — perhaps a metabolic drain induces secondary compensatory mutations. This possibility awaits experimental analysis through the performance of complementation experiments (genetic or with pure, synthetic AI-2) in many of the bacteria studied to date.

LuxS also represents an interesting subject for evolutionary studies. Generally, in the absence of LuxS and Pfs, the alternative enzyme, SahH, is present. However, both LuxS/Pfs and SahH can be absent (although this occurs predominantly in obligately host-associated species such as symbionts or parasites), and there is only one example (*Bifidobacterium*) in which all three proteins (LuxS, Pfs and SahH) are present. The linkage of *luxS* to other genes involved in the AMC and methionine synthesis indicates that LuxS arose through its role in metabolism, and further clues that relate to AI-2 function will probably be obtained from establishing the signal transduction/uptake pathways present in different bacteria (BOX 2).

Construction of strains that do not have a mechanism to sense or respond to exogenous AI-2 will permit dissection of the metabolic and signalling roles of LuxS. Results from the use of purified culture filtrates might have been misleading, as they contain a complex mixture of other signals to which bacteria could respond. Although strains that are deficient in an AI-2 response can currently be built in some organisms, further study of AI-2 export and inactivation mechanisms is required to reveal the processes involved.

LD_{50}
Dose lethal to 50% of animals tested.

Deciphering the true identity of DPD derivatives (that is, specific furanones), their pattern of production, functionality and relative abundance in different environments will provide evidence for the most physiologically relevant forms, and facilitate application of individual signalling molecules to discern their effects.

As explained, *in silico* analysis has unearthed alternative routes to achieve a complete AMC, and understanding the basis for this segregation will shed light on their roles. Moreover, genetic exchanges will enable formerly LuxS-bearing bacteria to harbour an entire AMC, although lacking the propensity to produce AI-2, or confer AI-2-producing ability upon organisms that would not normally do so. To fully appreciate the influence of LuxS, AI-2 and the AMC, a complete understanding of their effect on bacterial physiology, and the long-term effects of the absence of *luxS* on the survival of pathogens is needed.

The ultimate goal of such studies is the development of effective anti-microbial strategies, and there is already interest in the potential of anti-quorum sensing agents[5,108,109]. The propensity for quorum sensing to regulate virulence factors that are not essential for bacterial survival in the environment offers the attractive possibility that drugs directed to this mechanism will not exert a selective pressure and therefore resistance is less likely to emerge. Potential strategies include the use of molecules with a similar structure to autoinducers which interfere with signalling (such as furanones), and development of autoinducer degradation pathways[5,109]. Whereas the widespread distribution of AI-2 potentially offers the possibility to develop effective broad-range antimicrobials, both synthetic and natural molecules have been reported to interfere with AI-2 signalling[110,111], and there are mechanisms to take up and degrade AI-2 (REFS 46,47). Therefore, LuxS and AI-2 do not necessarily represent an ideal target for antimicrobials. Despite the dramatic phenotypic changes associated with AI-2 absence in *Vibrio* spp., other organisms do not show such pronounced effects, and in many cases those reported might be strain-specific. Perhaps the search could be more profitably focused on Pfs, which has been described as "an excellent target for broad-spectrum antimicrobial drug design"[112]. The inactivation of this gene causes more generally observed growth defects owing to its function in various prokaryotic cellular processes, including biological methylation, polyamine biosynthesis, methionine recycling, as well as bacterial quorum sensing[113].

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Competing interests statement

The authors declare no competing financial interests.

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DATABASES

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Entrez: http://www.ncbi.nlm.nih.gov/Entrez
Actinobacillus actinomycetemcomitans | *Bifidobacterium longum* | *Borrelia burgdorferi* | *Campylobacter jejuni* | *Clostridium perfringens* | *Escherichia coli* K-12 | *flaA* | *Helicobacter pylori* | *Neisseria meningitidis* | *Porphyromonas gingivalis* | *Salmonella enterica* serovar Typhimurium | *Shigella flexneri* | *speB* | *Streptococcus gordonii* | *Streptococcus mutans* | *Streptococcus pneumoniae* | *virB*
SwissProt: http://www.expasy.ch
SpeB | streptolysin S | VirB | VirF

FURTHER INFORMATION

Agnès Vendeville and Christoph Tang's laboratory:
http://www.imperial.ac.uk/cmmi
Kim Hardie, Klaus Winzer and Karin Heurlier's laboratory:
http://www.nottingham.ac.uk/iii
The Sanger Centre: http://genome.jgi-psf.org/microbial/index.html
TIGR: http://genome.jgi-psf.org/microbial/index.html
NCBI: http://www.ncbi.nlm.nih.gov/sutils/genom_table.cgi

SUPPLEMENTARY INFORMATION

See online article: S1 (figure)
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