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Look who's talking: communication and quorum sensing in the bacterial world

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For many years bacteria were considered primarily as autonomous unicellular organisms with little capacity for collective behaviour. However, we now appreciate that bacterial cells are in fact, highly communicative. The generic term 'quorum sensing' has been adopted to describe the bacterial cell-to-cell communication mechanisms which co-ordinate gene expression usually, but not always, when the population has reached a high cell density. Quorum sensing depends on the synthesis of small molecules (often referred to as pheromones or autoinducers) that diffuse in and out of bacterial cells. As the bacterial population density increases, so does the synthesis of quorum sensing signal molecules, and consequently, their concentration in the external environment rises. Once a critical threshold concentration has been reached, a target sensor kinase or response regulator is activated (or repressed) so facilitating the expression of quorum sensing-dependent genes. Quorum sensing enables a bacterial population to mount a co-operative response that improves access to nutrients or specific environmental niches, promotes collective defence against other competitor prokaryotes or eukaryotic defence mechanisms and facilitates survival through differentiation into morphological forms better able to combat environmental threats. Quorum sensing also crosses the prokaryotic–eukaryotic boundary since quorum sensing-dependent signalling can be exploited or inactivated by both plants and mammals.

Keywords: quorum sensing; cell-to-cell-communication; *N*-acylhomoserine lactones; autoinducers; bacteria; signalling

1. INTRODUCTION

> I think that a multiple of bacteria are stronger than a few and thus by union are able to overcome obstacles too great for the few. (Smith 1905)

In the unicellular bacterial world where each individual cell reproduces by binary fission and strives to out-compete its neighbours, recognition and co-operation between cells may at first appear very unlikely. Indeed Francois Jacob (1973) stated that 'It is perfectly possible to imagine a rather boring Universe without sex, without hormones and without nervous systems peopled only by individual cells reproducing *ad infinitum*. This Universe in fact exists. It is the one formed by a culture of bacteria.' However, there are many situations where the ability of a bacterial population to behave co-operatively and to recognize self from non-self could be highly advantageous particularly in the contexts of sex (conjugation), symbiosis and niche adaptation, production of secondary metabolites (e.g. antibiotics), combating the defence mechanisms of higher organisms and for facilitating population migration where the prevailing conditions in a specific environmental niche have become unfavourable.

Apart from direct cell–cell contact, the production of small diffusible chemicals probably offers the most obvious strategy for communication between bacterial cells. Such signal molecules could be considered as 'pheromones', a term originally coined by Karlson & Luscher (1959) from the Greek 'pherein' (to transfer) and 'hormon' (to excite). In contrast to hormones (which function as signals within a single organism), pheromones are secreted outside the producer organism and facilitate communication between individual organisms.

Bacteria release a wide variety of small molecules including secondary metabolites such as antibiotics and siderophores (iron chelators), metabolic end products and cell-to-cell signalling molecules which function as pheromones and are sometimes termed 'autoinducers' (where they function in part to stimulate their own synthesis). In many instances, the latter are considered to provide the bacterial population with a means of determining its numerical size (or density). As the bacterial culture grows, signal molecules are released into the extracellular milieu and accumulate. Once a threshold concentration of the molecule (and consequently a specific population density) is achieved, a co-ordinated change in bacterial behaviour is initiated. Fuqua *et al.* (1994) introduced the term 'quorum sensing' to describe this phenomenon, and since the early 1990s there has been an exponential increase in the number of published papers presenting new data on the nature and function of quorum sensing systems in diverse bacterial genera.

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One contribution of 12 to a Theme Issue 'Bacterial conversations: talking, listening and eavesdropping'.

The term quorum sensing does not, however, adequately describe all situations where bacteria employ diffusible chemical signals. The size of the quorum, for example, is not fixed but will vary according to the relative rates of production and loss of signal molecule, i.e. it is dependent on the prevailing local environmental conditions. It is also possible for a single bacterial cell to switch from the 'non-quorate' to the 'quorate' state as has been observed for *Staphylococcus aureus* trapped within an endosome in endothelial cells (Qazi *et al.* 2001). In this context, 'diffusion sensing' or 'compartment sensing' are more appropriate terms since the signal molecule is supplying information with respect to the local environment rather than cell population density *per se* (Redfield 2002; Winzer *et al.* 2002*b*). Quorum sensing might therefore be better considered as a special category of diffusion sensing where, in a given environment, the threshold concentration of signal molecule required to trigger a response can only be achieved by more than one cell (Redfield 2002; Winzer *et al.* 2002*b*). Furthermore, it should be remembered that quorum sensing, as the determinant of cell population density, is only one of many different environmental signals (e.g. temperature, pH, osmolarity, oxidative stress, nutrient deprivation) which bacterial cells must integrate in order to determine their optimal survival strategy (Withers *et al.* 2001). Thus, quorum sensing is an integral component of the global gene regulatory networks which are responsible for facilitating bacterial adaptation to environmental stress. Here, an overview of the current status of quorum sensing systems in Gram negative and Gram positive bacteria will be presented.

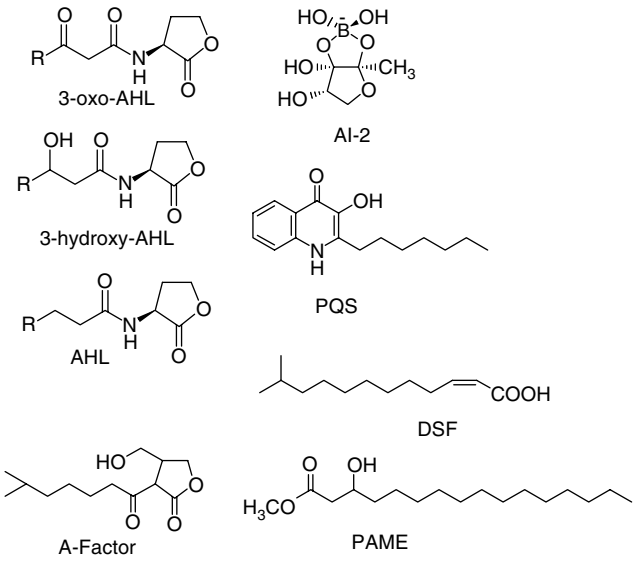

Figure 1. Structures of some representative quorum sensing signalling molecules. 3-oxo-AHL, *N*-(3-oxoacyl)homoserine lactone; 3-hydroxy-AHL, *N*-(3-hydoxyacyl)homoserine lactone and AHL, *N*-acylhomoserine lactone where R ranges from C1 to C15. The acyl side chains may also contain one or more double bonds: A-factor, 2-isocapryloyl-3-hydroxymethyl-γ-butyrolactone; AI-2, autoinducer-2, furanosyl borate ester form; PQS, *Pseudomonas* quinolone signal, 2-heptyl-3-hydroxy-4(1*H*)-quinolone; DSF, 'diffusible factor', methyl dodecenoic acid; PAME, hydroxyl-palmitic acid methyl ester.

2. DISCOVERING CELL-TO-CELL COMMUNICATION IN BACTERIA

The origins of the quorum sensing field can be traced back some four decades where several seminal papers on pheromone-like systems in bacteria hinted at the intriguing possibility that individual bacterial cells had ambitions beyond dividing into two and, in fact, that communication and co-operation were commonplace in the prokaryotic world. Indeed the paradigm of bacterial unicellular existence was challenged by the early work on fruiting body formation in *Myxococcus xanthus* (McVittie *et al.* 1962), on streptomycin biosynthesis and aerial mycelium formation in *Streptomyces griseus* (Khoklov *et al.* 1967), on the induction of genetic competence in *Streptococcus pneumoniae* (Tomasz 1965) and on the control of bioluminescence in marine vibrios (Nealson *et al.* 1970). With respect to the last, it was noted that although *Vibrio fischeri* only produced light at high cell population densities, the organism produced an extracellular substance that could induce bioluminescence at low cell densities. The 'autoinducer' concerned was subsequently purified and the structure determined as *N*-(3-oxohexanoyl)homoserine lactone (3-oxo-C6-HSL) (Eberhard *et al.* 1981). Until 1992, this *N*-acylhomoserine lactone (AHL) was exclusively associated with *V. fischeri*. However, while working on the biosynthesis of the β-lactam antibiotic, carbapen-2-em-3-carboxylic acid in the plant pathogen *Erwinia carotovora*, Bainton *et al.* 1992*a*,*b*) discovered that carbapenem biosynthesis in this terrestrial microbe was also regulated by 3-oxo-C6-HSL and that other Gram negative bacteria including *Pseudomonas aeruginosa* and *Serratia marcescens* also produced the *V. fischeri* autoinducer. This work was rapidly followed by numerous papers reporting the presence of AHLs in a variety of different Gram negative bacteria and their role in regulating virulence and plasmid transfer as well as bioluminescence and antibiotic biosynthesis (Jones *et al.* 1993; Passador *et al.* 1993; Swift *et al.* 1993; Zhang *et al.* 1993). Subsequently this resulted in the introduction of the term 'quorum sensing' (Fuqua *et al.* 1994) to describe AHL-dependent bacterial cell-to-cell communication. AHLs (figure 1) are, however, not the only class of quorum sensing signal molecule. In Gram negative bacteria, 4-quinolones, fatty acids and fatty acid methyl esters have been identified as quorum sensing signal molecules (figure 1). Apart from γ-butyrolactones such as Khoklov's A-factor produced by *Streptomyces* (figure 1), Gram positive bacteria employ unmodified (e.g. the competence stimulating factors of *S. pneumoniae*) or post-translationally modified peptides such as the staphylococcal cyclic peptides (figure 2). Although no 'universal' bacterial quorum sensing system or signal molecule family has yet been discovered, many Gram negative and Gram positive bacteria produce 'autoinducer-2' (figure 1), a collective term for a family of interconvertible furanone compounds.

Given the vast number of extracellular metabolites, the chemical diversity among known quorum sensing signal molecules is likely to represent only the 'tip of the

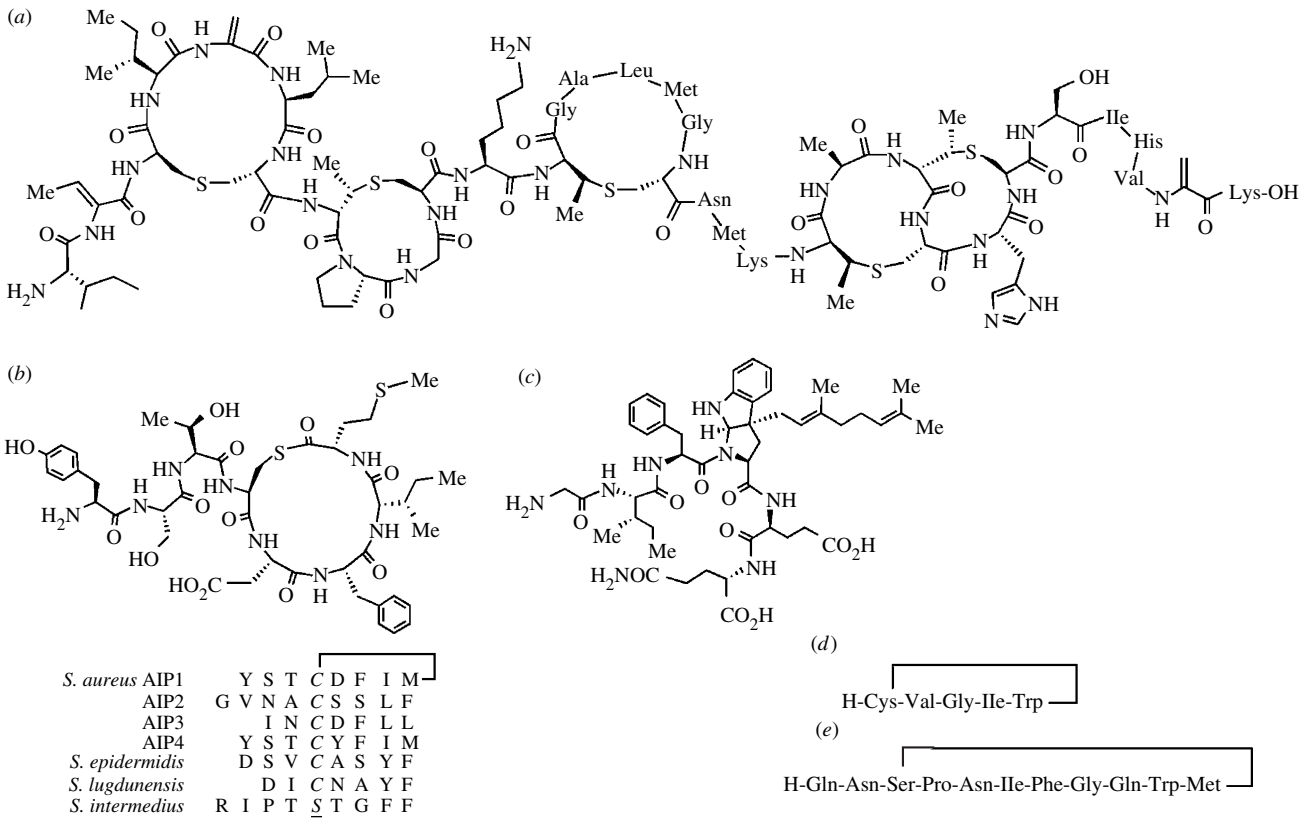

Figure 2. Chemical structures of the quorum sensing signal molecules: (*a*) nisin from *L. lactis*; (*b*) (*top*) autoinducing peptide-1 (AIP-1) from *S. aureus* and (*bottom*) schematic structures of characterized staphylococcal AIPs; (*c*) ComX from *B. subtilis* RO-E-2; (*d*) AIP from *Lactobacillus plantarum*; and (*e*) 28-membered AIP from *E. faecalis*.

iceberg'. Indeed, Yim *et al.* (2006) have argued that that the majority of low-molecular-weight organic compounds made and secreted by microbes are likely to function as cell-signalling molecules which modulate the metabolic activities of natural microbial communities. In particular, they have presented a persuasive argument that antibiotics evolved as signal molecules given that sub-growth-inhibitory concentrations are potent modulators of gene expression. Although antibiotics can clearly signal, whether they are involved in cell-to-cell communication, i.e. can be considered as quorum sensing signal molecules is not clear. Quorum sensing is generally considered in the context of cell-to-cell signalling between members of the same bacterial species rather than as a response of one organism to a metabolite produced by another. Nevertheless antibiotics possess many of the characteristic features of quorum sensing signal molecules which require that: (i) the production of the quorum sensing signal takes place during specific stages of growth, under certain physiological conditions, or in response to environmental changes; (ii) the quorum sensing signal accumulates in the extracellular milieu and is recognized by a specific bacterial receptor; (iii) the accumulation of a critical threshold concentration of the quorum sensing signal generates a concerted response and (iv) the cellular response extends beyond physiological changes required to metabolize or detoxify the molecule (Winzer *et al.* 2002*b*).

Unless all four criteria are met, a molecule cannot really be classified as a quorum sensing signal molecule, given that many extracellular bacterial metabolites meet the first three. Examples include toxic bacterial metabolites which accumulate and trigger a coordinated stress response once they reach a critical concentration. Such metabolites cannot be considered as intercellular communication signals, as the cells are merely responding to the toxicity of the molecule. Similarly, other metabolites can induce, during their release, their own uptake systems and the production of enzymes required for their breakdown. This may indirectly influence the expression of genes from other linked metabolic pathways and emphasizes the importance of criterion (iv) when assigning a quorum sensing function to a given molecule.

3. ACYLHOMOSERINE LACTONE-DEPENDENT QUORUM SENSING IN GRAM-NEGATIVE BACTERIA

AHL-mediated quorum sensing is employed by diverse Gram negative proteobacteria belonging to α, β and γ subdivisions, but no AHL-producing Gram positive bacteria have so far been identified (Swift *et al.* 1998; Withers *et al.* 2001; Cámara *et al.* 2002*b*; Chhabra *et al.* 2005) (table 1). Numerous AHL biosensor assays based on *lux*, *gfp* or *lacZ* reporter gene fusions (Bainton *et al.* 1992*a*; Shaw *et al.* 1997; Winson *et al.* 1998; Andersen *et al.* 2001) or pigment induction (e.g. violacein in *Chromobacterium violaceum* (McClean *et al.* 1997) have been developed and these have greatly simplified and facilitated screening for AHL production. These assays, however, only provide tentative identification and confirmation of chemical identity requires mass spectrometry and nuclear magnetic

Table 1. Some examples of LuxR/LuxI/AHL-dependent quorum sensing systems in Gram-negative bacteria. (The structures of some of these signals and the definitions of the abbreviations used are in figures 1 and 2.)

organism	major AHL(s)	LuxR	LuxI	phenotypes
Aeromonas hydrophila	C4-HSL	AhyR	AhyI	biofilms, exoproteases
Aeromonas salmonicida	C4-HSL	AsaR	AsaI	exoprotease
Agrobacterium tumefaciens	3-oxo-C8-HSL	TraR	TraI	plasmid conjugation
Agrobacterium vitiae	C14:1-HSL, 3-oxo-C16:1-HSL	AvsR	AvsI	virulence
Burkholderia cenocepacia	C6-HSL, C8-HSL	CepR, CciR	CepI, CciI	exoenzymes, biofilm formation, swarming motility, siderophore, virulence
Burkholderia pseudomallei	C8-HSL, C10-HSL, 3-hydroxy-C8-HSL, 3-hydroxy-C10-HSL, 3-hydroxy-C14-HSL	PmlIR1, BpmR2, BpmR3	PmlI1, PmlI2, PmlI3	virulence, exoprotease
Burkholderia mallei	C8-HSL, C10-HSL	BmaR1, BmaR3, BmaR4, BmaR5	BmaI1, BmaI3	Virulence
Chromobacterium violaceum	C6-HSL	CviR	CviI	exoenzymes, cyanide, pigment
Erwinia carotovora ssp. *carotovora*	3-oxo-C6-HSL	ExpR/CarR	CarI (ExpI)	carbapenem, exoenzymes, virulence
Pantoea (*Erwinia*) *stewartii*	3-oxo-C6-HSL	EsaR	EsaI	exopolysaccharide
Pseudomonas aeruginosa	C4-HSL; 3-oxo-C12-HSL	LasR, RhlR, QscR, VqsR	LasI, RhlI	exoenzymes, secretion, HCN, biofilms
Pseudomonas aureofaciens	C6-HSL	PhzR, CsaR	PhzI, CsaI	phenazines, protease, colony morphology, aggregation
Pseudomonas putida	3-oxo-C10-HSL, 3-oxo-C12-HSL	PpuR	PpuI	biofilm formation
Pseudomonas chlororaphis	C6-HSL	PhzR	PhzI	phenazine-1-caboxamide
Pseudomonas syringae	3-oxo-C6-HSL	AhlR	AhlI	exopolysaccharide, swimming motility, virulence
Rhizobium leguminosarum bv *viciae*	7-*cis*-C14-HSL/C6-HSL/C7-HSL/C8-HSL, 3-oxo-C8-HSL, 3-hydroxy-C8-HSL	CinR, RhiR, RaiR, TraR, BisR, TriR	CinI, RhiI, RaiI	root nodulation/symbiosis, plasmid transfer, growth inhibition; stationary phase adaptation
Rhodobacter sphaeroides	7-*cis*-C14-HSL	CerR	CerI	aggregation
Serratia spp. ATCC 39006	C4-HSL	SmaR	SmaI	antibiotic, pigment, exoenzymes
Serratia liquefaciens MG1	C4-HSL	SwrR	SwrI	swarming motility, exoprotease, biofilm development, biosurfactant
Serratia marcescens SS-1	C6-HSL, 3-oxo-C6-HSL	SpnR	SpnI	sliding motility, biosurfactant, pigment, nuclease, transposition frequency
Serratia proteamaculans B5a	3-oxo-C6-HSL	SprR	SprI	exoenzymes
Sinorhizobium meliloti	C8-HSL, C12-HSL, 3-oxo-C14-HSL, 3-oxo-C16:1-HSL, C16:1-HSL, C18-HSL	SinR, ExpR, TraR	SinI	nodulation/symbiosis
Vibrio fischeri	3-oxo-C6-HSL	LuxR	LuxI	bioluminescence
Yersinia enterocolitica	C6-HSL, 3-oxo-C6-HSL, 3-oxo-C10-HSL, 3-oxo-C12-HSL, 3-oxo-C14-HSL	YenR, YenR2	YenI	swimming and swarming motility
Yersinia pseudotuberculosis	C6-HSL, 3-oxo-C6-HSL, C8-HSL	YpsR, YtbR	YpsI, YtbI	motility, aggregation

resonance (NMR) spectroscopy (Chhabra *et al.* 2005). Nevertheless, AHL biosensors have usefully been used to examine both terrestrial, freshwater and marine environments for AHL producers (Elasri *et al.* 2001; Wagner-Döbler *et al.* 2005). For example, in a survey of soil and plant-associated *Pseudomonas* species, AHL production was most commonly found in plant-associated bacteria, leading to the suggestion that AHL production may occur more frequently among bacteria living in close association with higher organisms (Elasri *et al.* 2001). However, many obligate Gram negative human pathogens (e.g. *Neisseria meningitidis*, *Haemophilus influenzae*, *Helicobacter pylori*) do not produce AHLs (Swift *et al.* 1998). Although many members of the Enterobacteriaceae are AHL producers this is, perhaps surprisingly, not the case for either *Escherichia coli* or *Salmonella*.

Most AHL-producers synthesize multiple AHLs which are characterized by a homoserine lactone (HSL) ring unsubstituted in the β- and γ-positions which is *N*-acylated with a fatty acyl group at the α-position (figure 1). The acyl chain varies in length,

saturation level and oxidation state. In most cases the chain has even number of carbons (C4–C18) although AHLs with C5 and C7 acyl chains have been identified (Lithgow *et al.* 2000; Horng *et al.* 2002). Examples of different AHLs produced by Gram-negative bacteria are shown in table 1. They belong either to the *N*-acyl, *N*-(3-oxoacyl) or *N*-(3-hydroxyacyl) classes of compounds (figure 1; Chhabra *et al.* 2005). AHLs with C14 and C18 acyl chains have also been described which also contain one or two double bonds (Schripsema *et al.* 1996; Wagner-Döbler *et al.* 2005).

AHL-mediated signalling appears to require at least a C4 acyl side chain since *N*-butanoylhomoserine lactone (C4-HSL) and *N*-hydroxybutanoylhomoserine lactone (3-hydroxy-C4-HSL) are the shortest AHLs found naturally (Cao & Meighen 1989; Winson *et al.* 1995). This is probably because the HSL ring is highly susceptible to pH-dependent ring opening, a susceptibility which decreases as the acyl side chain is lengthened (Yates *et al.* 2002). Consequently, HSL and *N*-propionylhomoserine lactone (C3-HSL) are rapidly hydrolysed at pHs well below 7.0. The HSL ring for example, is largely open when the pH is raised from 1 to 2. By introducing a C3 acyl chain (C3-HSL), the ring remains largely intact at pH 2 but around 70% is hydrolysed by pH 6, in contrast to C4-HSL which is only completely ring-opened at pH 8 (Yates *et al.* 2002). Ring-opened AHLs are not active as quorum sensing signal molecules. Given the stability of the HSL ring at acidic pHs, it is perhaps not too surprising that the acidophilic extremophile, *Acidithiobacillus ferrooxidans* employs AHL dependent quorum sensing (Farah *et al.* 2005). This organism is involved in the bioleaching of metal sulphide ores and produces at least nine AHLs including *N*-acyl, *N*-(3-oxoacyl) and *N*-(3-hydroxyacyl) compounds ranging from C8–C16 in acyl chain length (Farah *et al.* 2005).

(a) *Acylhomoserine lactone-mediated signal synthesis and transduction*

The central components of AHL-driven quorum sensing systems are typically members of the LuxI and LuxR protein families (Fuqua *et al.* 2001; Swift *et al.* 2001). AHLs diffuse across the bacterial cell envelope and subsequently accumulate in the surrounding milieu. Once a sufficient AHL concentration has been attained within the culture, AHLs bind to and activate members of the LuxR transcriptional regulator protein family. The LuxR/AHL complex is responsible for activating or repressing multiple target structural genes (Fuqua *et al.* 2001; Swift *et al.* 2001). In many cases, the *luxI* gene is a target for the activated LuxR/AHL complex, resulting in a positive autoinduction circuit in which the AHL signal molecule also controls its own synthesis (figure 3). Furthermore, many bacteria (e.g. *P. aeruginosa*, *Yersinia pseudotuberculosis*, *Burkholderia pseudomallei* and *Rhizobium leguminosarum*) possess multiple LuxR/LuxI/AHL modules (table 1) which are often interdependent.

Phylogenetic comparisons of LuxI–LuxR family members have highlighted the possibility that one or more of these quorum sensing systems may have been acquired by lateral gene transfer (Gray & Garey 2001). Indeed a number of LuxR and LuxI homologues are

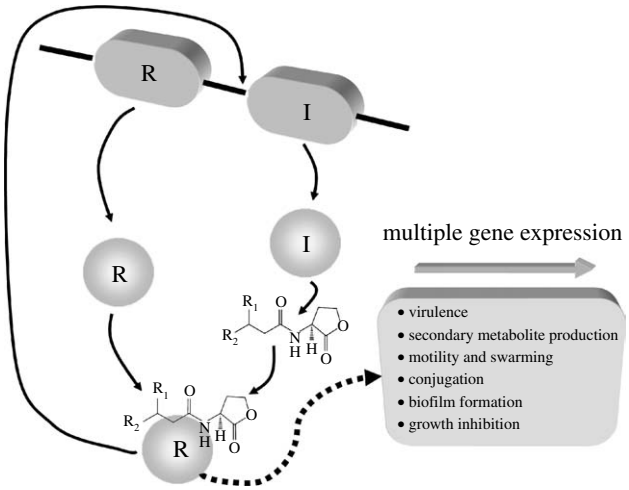

Figure 3. LuxR/AHL-driven quorum sensing module where LuxR is the AHL receptor and signal transducer and LuxI is the AHL signal synthase. Many bacteria possess multiple LuxR/LuxI/AHL modules.

located on plasmids such as the *Agrobacterium* Ti plasmid (Zhang *et al.* 1993) and *Rhizobium* Symbiotic plasmids (Wisniewski-Dye & Downie 2003). Recently Wei *et al.* (2006) discovered a LuxRI system in *S. marcescens* termed SpnRI which is located on a functional Tn3 type transposon (Tn*TIR*) and could be mobilized between plasmids and chromosomes in *E. coli* and *Serratia*. Acquisition of the SpnR by an AHL-negative *Serratia* strain resulted in the AHL-dependent regulation of swarming motility and pigment biosynthesis. These data suggest that lateral gene transfer might well play an important role in the transfer of LuxRI modules between different bacterial genera (Wei *et al.* 2006).

AHLs are usually synthesized via members of the LuxI protein family which use the appropriately charged acyl-acyl carrier protein (acyl-ACP) and *S*-adenosylmethionine as the sources of the acyl side chain and the HSL ring moiety, respectively (Moré *et al.* 1996; Jiang *et al.* 1998; Parsek *et al.* 1999). The bacterial genome databases now contain more than 100 different LuxI homologues, many of which show low protein sequence homologies but have ten invariant residues in the amino terminal half of the protein (Fuqua *et al.* 2001). Furthermore, phylogenetic comparisons do not facilitate prediction of the nature of the AHL(s) synthesized via a given LuxI homologue. The crystal structures of two LuxI homologous proteins, EsaI and LasI, have been solved and both proteins belong to the GCN5-related *N*-acetyltransferase protein family (Watson *et al.* 2002; Gould *et al.* 2004). From the structural analysis, threonine at position 140 was shown to contribute to the specificity of EsaI for 3-oxo-acyl-ACPs but not to be of such importance for LasI (Watson *et al.* 2002; Gould *et al.* 2006).

The biosynthesis of AHLs is not exclusively dependent on LuxI homologues. The LuxM family of AHL synthases, originally discovered in *Vibrio harveyi* (Bassler *et al.* 1993), has also been found in other *Vibrio* species (Hanzelka *et al.* 1999; Milton *et al.* 2001). Despite the complete lack of amino acid sequence homology with the LuxI family, LuxM proteins such as AinS catalyse AHL formation using the same

substrates as LuxI proteins (Hanzelka *et al.* 1999). Interestingly, LuxM and LuxI homologues have been shown to co-exist in some vibrios. This is the case for both *V. fischeri* (AinS and LuxI; Hanzelka *et al.* 1999) and *Vibrio anguillarum* (VanI and VanM; Milton *et al.* 2001). In contrast to most *luxI* genes, *luxM* genes are not genetically linked to cognate transcriptional regulators: instead they are associated with genes coding for histidine protein kinase sensors which, upon interaction with AHLs in the periplasm, trigger a phosphorelay cascade leading to transcriptional activation of the target quorum sensing dependent genes (Cámara *et al.* 2002*a*; Croxatto *et al.* 2004). A third potential AHL synthase (HdtS) which does not belong to either the LuxI or LuxM families has been identified by Laue *et al.* (2000).

Although no AHL-producing strains of *E. coli* or *Salmonella* have been identified, both possess a LuxR-homologue termed SdiA (Ahmer 2004). Expression of SdiA-regulated genes cannot be activated by the addition of spent culture supernatants from *E. coli* or *Salmonella* strains. However, they can be activated in the presence of certain AHL-producing bacteria or most sensitively by the exogenous provision of either 3-oxo-C6-HSL or 3-oxo-C8-HSL (Michael *et al.* 2001). Recent NMR studies using the purified N-terminal domain of SdiA from *E. coli* showed that these proteins are subject to a folding-switch in the presence of AHLs, demonstrating the ability of AHLs to interact with SdiA and induce a conformational change (Yao *et al.* 2006). The fact that this type of signalling does not result in two-way communication has led to speculation that SdiA is employed for signal interception (Ahmer 2004).

(b) *The hierarchical quorum sensing circuitry of* P. aeruginosa

In different Gram negative bacteria, AHL-dependent quorum sensing circuitries control the expression of genes involved in secondary metabolite production, plasmid transfer, bioluminescence, motility, biofilm maturation, and virulence (table 1). One of the most intensively investigated quorum sensing systems is that present in the opportunistic human pathogen *P. aeruginosa* which integrates AHL-dependent signalling with 4-quinolone dependent quorum sensing. At least 6% (over 300 genes) of the 6.3 MB *P. aeruginosa* genome is AHL-regulated via the *las* and *rhl* quorum sensing systems (Hentzer *et al.* 2003; Schuster *et al.* 2003; Wagner *et al.* 2004). These consist of the LuxRI homologues, LasRI (Gambello & Iglewski 1991; Passador *et al.* 1993) and RhlRI (Latifi *et al.* 1995), respectively. LasI directs the synthesis of primarily *N*-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-C12-HSL; Pearson *et al.* 1994) and together with the LasR regulates the production of, for example, virulence factors including elastase, the LasA protease, alkaline protease and exotoxin A (Gambello & Iglewski 1991; De Kievit & Iglewski 2000). RhlI directs the synthesis of C4-HSL (Latifi *et al.* 1995; Winson *et al.* 1995), which activates RhlR and in turn induces, for example, the production of rhamnolipids, elastase, LasA protease, hydrogen cyanide, pyocyanin, siderophores and the LecA and LecB lectins (Latifi *et al.* 1995; Winson *et al.* 1995; Latifi *et al.* 1996; Winzer *et al.* 2000; Diggle *et al.* 2002). Mutation of either or both the *las* and *rhl* systems results in attenuation of virulence with a *lasI rhlI* mutant being the most highly attenuated strain in a mouse model of pneumonia (Pearson *et al.* 2000); a loss of swarming motility (*rhl*) (Diggle *et al.* 2002), and marked changes in biofilm architecture (both *las* and *rhl*). Furthermore, the loss of quorum sensing in *P. aeruginosa* biofilms by mutation or administration of quorum sensing inhibitory agents renders the biofilm much more susceptible to hydrogen peroxide, to antibiotics such as tobramycin and to phagocytic cells (Bjarnsholt *et al.* 2005).

The *las* and the *rhl* systems are organized hierarchically such that the *las* system exerts transcriptional control over both *rhlR* and *rhlI* (Latifi *et al.* 1996). However, the expression of *rhlRI* is not exclusively dependent on a functional *las* system. For *P. aeruginosa* genes such as *lecA*, expression in a *lasR* mutant is delayed rather than abolished (Winzer *et al.* 2000). Transcriptome studies (Schuster *et al.* 2003; Wagner *et al.* 2004) have revealed that the *las* and *rhl* regulated genes and operons are scattered throughout the chromosome, supporting the view that the *P. aeruginosa* quorum sensing circuitry constitutes a global regulatory system (Schuster & Greenberg 2006). The *las*/*rhl* system incorporates further levels of complexity introduced by the presence of two additional LuxR homologues, termed QscR and VqsR, which are not genetically linked to an AHL synthase gene (Chugani *et al.* 2001). QscR represses *lasI* expression earlier in growth, possibly through the formation of inactive heterodimers with LasR and RhlR (Chugani *et al.* 2001; Ledgham *et al.* 2003). When AHL concentration increases it is thought that these heterodimers dissociate, facilitating the formation of active LasR and RhlR homo-dimers which can then activate quorum sensing-mediated gene expression. Recently it has been shown that QscR activation requires 3-oxo-C12-HSL although it does exhibit a relaxed AHL specificity when compared with LasR. This suggests that QscR may also respond to AHLs made by other bacteria within mixed bacterial populations (Lee *et al.* 2006). VqsR also plays an important role in the positive regulation of virulence and quorum sensing in *P. aeruginosa*, since a mutation in this regulator results in the abolition of AHL and extracellular virulence factor production with a subsequent reduction in pathogenicity in a nematode infection model (Juhas *et al.* 2004). This positive VqsR-dependent regulation is mediated via *lasI* (Juhas *et al.* 2004, 2005). Futhermore, transcriptome analysis has revealed that a high proportion of the genes regulated by VqsR are also regulated by AHL-dependent quorum sensing, demonstrating the close association between this LuxR-homologue and the *las* and *rhl* circuitry (Juhas *et al.* 2004).

In *V. fischeri* and *E. carotovora*, provision of the exogenous cognate AHL overcomes the cell population density dependent induction of bioluminescence and carbapenem production, respectively (Nealson *et al.* 1970; Williams *et al.* 1992). Such responses to exogenously supplied AHLs were initially considered to be characteristic of quorum sensing systems. However, for bacteria such as *P. aeruginosa*,

the provision of either or both 3-oxo-C2-HSL and C4-HSL does not overcome the cell population density dependence of quorum sensing regulated genes such as *lecA* (Winzer *et al.* 2000; Diggle *et al.* 2002). This is primarily because both the quorum sensing modules themselves and their target genes are subject to additional layers of regulation mediated at both the transcriptional and post-transcriptional levels (Venturi 2006). Such regulators include: Vfr, the cAMP receptor regulatory protein (Albus *et al.* 1997; Beatson *et al.* 2002), the stationary-phase sigma factor RpoS (Schuster *et al.* 2004), the alternative sigma factor RpoN (Heurlier *et al.* 2003), the stringent response protein RelA (Van Delden *et al.* 2001; Erickson *et al.* 2004), the post-transcriptional regulators RsmA (Pessi *et al.* 2001; Heurlier *et al.* 2004) and DksA (Jude *et al.* 2003), the transcriptional regulators RsaL (De Kievit *et al.* 1999; Rampioni *et al.* 2006), MvaT (Diggle *et al.* 2002), the anaerobic regulator ANR (Pessi & Haas 2000), the AraC-type regulator VqsM (Dong *et al.* 2005*a*) and two members of the two component signal transduction response regulator family, namely GacA (Reimmann *et al.* 1997) and PrpB (Dong *et al.* 2005*b*), respectively. The *rhl* system has also been reported to be up-regulated in response to interferon-γ binding to the major *P. aeruginosa* outer membrane protein OprF (Wu *et al.* 2005*a*), although the mechanism by which this host cytokine regulates quorum sensing is not yet known.

The *las* and *rhl* hierarchy is also linked to a further quorum sensing system which employs a chemically distinct signal molecule, 2-heptyl-3-hydroxy-4(1*H*)-quinolone, the Pseudomonas quinolone signal (PQS; figure 1). This 4-quinolone is required for the production of *rhl*-dependent exoproducts at the onset of stationary phase (Pesci *et al.* 1999; Diggle *et al.* 2003) but, in contrast to the AHLs, is capable of overcoming the cell density but not growth phase-dependent production of *P. aeruginosa* exoproducts (Diggle *et al.* 2003). Interestingly, *P. aeruginosa* strains carrying mutations in the quorum sensing-regulated multi-drug efflux pump MexGHI-OpmD, are unable to produce wild type levels of PQS or AHLs and are severely attenuated in both mouse and plant experimental infection models, exhibit a growth defect and an altered antibiotic susceptibility profile. This pleiotropic phenotype could, however, be restored to wild type by supplying exogenous PQS (Aendenkerk *et al.* 2005). These data imply that the AHL/PQS-dependent quorum sensing regulatory network plays a central role in co-ordinating virulence, antibiotic resistance and fitness in *P. aeruginosa*.

4. PEPTIDE-MEDIATED QUORUM SENSING IN GRAM-POSITIVE BACTERIA

While Gram-negative bacteria employ hydrophobic low molecular weight signal molecules, post-translationally modified peptides are engaged by Gram-positive bacteria as quorum sensing signal molecules. These peptides, referred to as autoinducing peptides (AIPs), range from 5 to 34 amino acids in length and typically contain unusual chemical architectures. Based on their structural uniqueness (figure 2), three different families of AIPs are known to date: (i) the oligopeptide lantibiotics, typified by the lactococcal nisin, which are characterized by the presence of lanthionine-mediated thioether macrocyclic features and dehydroamino acid residues (Van der Meer *et al.* 1993; Quadri 2002); (ii) the 16-membered thiolactone peptides, exemplified by the staphylococcal AIP-1 (Ji *et al.* 1997; McDowell *et al.* 2001; Chan *et al* 2004); and (iii) the isoprenylated tryptophan peptides, in which ComX and its variants from *Bacillus subtilis* and other *Bacillus* species are currently the only known members (Ansaldi *et al.* 2002; Okada *et al.* 2005).

The lantibiotics, including nisin which is produced by *Lactococcus lactis*, display exceptionally potent bactericidal activities against a wide spectrum of Gram-positive organisms. The quorum sensing system mediated by lantibiotics is unique since it specifically regulates, in a cell population density-dependent manner, the biosynthesis of a potentially harmful signal molecule. For example, the biosynthesis of the autoinducer nisin requires a cluster of eleven genes, *nisABCTIPRKFEG*. It is a self-inducible system involving the two-component regulatory system NisK–NisR which controls the expression of proteins involved in nisin biosynthesis (NisABCP) and immunity (NisI) (Kuipers *et al.* 1995; Dodd *et al.* 1996). In contrast, the autoinducer ComX (figure 2) in *B. subtilis*, which is sensed by the two component system ComP–ComA, upregulates the expression of many genes required for competence development (Tortosa & Dubnau 1999). Recently, the chemical structure of the hexapeptide ComX from *B. subtilis* RO-E-2 was unambiguously established, in which the conserved tryptophan residue is modified by a geranyl group at the C^3 of the indole side-chain, as well as stereospecific intramolecular (indole $C^2 \rightarrow N^{\alpha}$) cyclization to give a rigid tricyclic structure. These unique post-translational modifications appear to be crucial for biological activity (Okada *et al.* 2005) and constitute key determinants in the interaction of ComX with its cognate receptor, ComP. It is anticipated that ComX from different strains of *B. subtilis*, which are engaged in quorum sensing, will contain these unique tryptophan modifications but may differ in the length of the peptide chain and the isoprenyl group (e.g. geranyl and farnesyl; Ansaldi & Dubnau 2004).

Quorum sensing mediated via thiolactone peptides is the most extensively studied system. This family of AIPs is structurally characterized by a 16-membered side chain-to-tail macrocyclic peptide to which is attached N-terminally a short linear peptide; the prototypical member of this family is the modified octapeptide AIP-1 (figure 2) employed by *S. aureus* (Ji *et al* 1995, 1997). The staphylococcal AIPs are derived from a polycistronic locus, *agrBDCA* that comprises the genes required for AIP synthesis (*agrBD*) and AIP response (*agrAC*). The thiolactone macrocyclic structure is enzymatically derived from an internal fragment of the precursor protein (AgrD) involving the condensation of a Cys sulphhydryl group to the C-terminal carboxylic acid; this unique post-translational modification is brought about by AgrB (Zhang *et al.* 2002, 2004). Several variants of

the staphylococcal signal molecule have been reported (Ji *et al.* 1997; McDowell *et al.* 2001; Chan *et al.* 2004), and while the primary amino acid sequences are different, they share a common central Cys that is located four residues from the C-terminal amino acid of the processed peptide (see figure 2). This family of AIPs exhibit two other common chemical features: (i) amino acid residues bearing aromatic and hydrophobic side chains are frequently located within the macrocyclic domain and (ii) the exocyclic peptide chain is usually hydrophilic. In fact, *S. aureus* strains can be divided into four groups (I–IV) on the basis of their ability to cross-activate or inhibit *agr* expression, e.g. AIP-2 is a potent inhibitor of *S. aureus* groups-I, -III and -IV quorum sensing systems (Mayville *et al.* 1999; Lyon *et al.* 2002). Functionally, the AIPs are sensed by the two-component signal transduction system (TCSTS) comprising AgrC, a transmembrane sensor kinase, and AgrA, a response regulatory protein. Interaction of the AIP with its cognate AgrC results in activation of the TCSTS, thus resulting in upregulation of the *agr*-mediated quorum sensing system. The effector of the *S. aureus* quorum sensing system is a 517 nucleotide transcript, RNAIII, which has the capacity to initiate the transcription of genes that encode a variety of exoproteins, e.g. *hla* (encoding α-haemolysin), *saeB* (enterotoxin B), *tst* (TSST-1), *ssp* and *spr* (serine proteases), and to repress the genes encoding cell surface proteins, e.g. *spa* (protein A) and *fnb* (fibronectin-binding protein) (Ji *et al.* 1995, 1997; Dunman *et al.* 2001; McDowell *et al.* 2001). Thus, the *agr* regulon effectively controls the balance of virulence factor expression during the colonization and invasion phases of the staphylococcal infection. In this respect, chemical agents that block quorum sensing in *S. aureus* (Lyon *et al.* 2002; Scott *et al.* 2003; Chan *et al* 2004) have recently been investigated for the treatment or management of acute staphylococcal infections. For example, near-complete attenuation of acute subcutaneous infections in mice were demonstrated when *S. aureus* group-I was co-administered with AIP-2 (Mayville *et al.* 1999).

Structural variants of *S. aureus* AIPs (see figure 2) have recently been characterized from *Staphylococcus epidermidis* (Otto *et al.* 1998, 2001; Carmody & Otto 2004), *Staphylococcus intermedius* (Kalkum *et al.* 2003; Ji *et al.* 2005), *Enterococcus faecalis* (Nakayama *et al.* 2001), *Lactobacillus plantarum* (Sturme *et al.* 2005) and *Listeria monocytogenes* (Autret *et al.* 2003). A pentapeptide thiolactone (LamD558) from *L. plantarum* has been chemically characterized and an *agr*-like operon identified (Sturme *et al.* 2005). However, although the *lamBDCA* operon regulates genes encoding surface polysaccharides, cell membrane proteins and sugar utilization proteins, LamD558 did not exhibit any *lam* autoregulatory activity and it is possible that a different AIP is involved in *lam* regulation (Sturme *et al.* 2005).

Interestingly, although displaying many of the distinctive characteristics of the family of 16-membered cyclic AIPs, an unexpected chemical feature of the *S. intermedius* AIP is the use of serine instead of cysteine as the critical residue to accomplish macrocyclization, thus giving rise to a lactone ring (Ji *et al.* 2005). The autoinducer identified from *E. faecalis*, which regulates production of the virulence factor gelatinase, is also strikingly different from the other members of this family of AIPs. Although cyclization is mediated by a serine residue, the ensuing lactone macrocyclic ring is 28-membered and involves nine amino acid residues.

This family of small-to-medium size thiolactone/lactone peptides clearly represents the principal chemical architecture utilized by Gram-positive bacteria to mediate quorum sensing. The differences in AIP primary sequence ensures a high degree of selectivity, matched by divergently different cognate sensors or receptors.

5. LuxS AND AI-2-MEDIATED QUORUM SENSING

The only presently known quorum sensing mechanism which appears to be shared by both Gram-positive and Gram-negative bacteria is based on a group of interconvertible, diffusible molecules collectively referred to as autoinducer-2 (AI-2). The LuxS protein required for the production of AI-2 (Schauder *et al.* 2001; Xavier & Bassler 2003) is an iron-containing enzyme (Zhu *et al.* 2003*a*) which cleaves *S*-ribosyl-L-homocysteine (SRH) to generate homocysteine and the AI-2 precursor, 4,5-dihydroxy-2,3-pentanedione (DPD). The latter cyclizes spontaneously and gives rise to a number of related furanone derivatives which are thought to be in equilibrium with each other (Miller *et al.* 2004). At least two of these are recognized by specific binding proteins in *Vibrio* spp. and *Salmonella enterica*, respectively. The *luxS* gene is widespread and presently found in over 60 species, including members of the β-, γ-, δ- and ε-proteobacteria, spirochaetales, firmicutes, and actinobacteridae, as well as genera belonging to the Deinococcus and Cytophaga groups, and the green sulphur bacteria (Vendeville *et al.* 2005), suggesting that AI-2 may form the basis of a widespread language used for interspecies communication (Xavier & Bassler 2003).

The LuxS/AI-2 system has been analysed in detail in *Vibrio* spp., in particular *V. harveyi* and *Vibrio cholerae*, where it is involved in the regulation of bioluminescence and virulence-associated traits, respectively (Miller *et al.* 2002; Xavier & Bassler 2003; Henke & Bassler 2004*a*,*b*; Lenz *et al.* 2004). The AI-2 molecule recognized by these species is a furanosyl borate diester (figure 1), which binds tightly to its receptor, the periplasmic binding protein LuxP. The resulting complex then interacts with a domain of the membrane-bound histidine kinase LuxQ (Neiditch *et al.* 2005), triggering a complex response which involves a phosphorelay system and small regulatory RNAs (Lenz *et al.* 2004). In *V. cholerae* and *V. harveyi*, this system also integrates the signals from other autoinducers and their corresponding sensor kinases (Miller *et al.* 2002; Henke & Bassler 2004*a*).

The precise role of AI-2 in other bacteria remains a matter of debate. Presently, outside of the genus *Vibrio*, the only definitive genes shown to be regulated by AI-2 are those involved in AI-2 uptake,

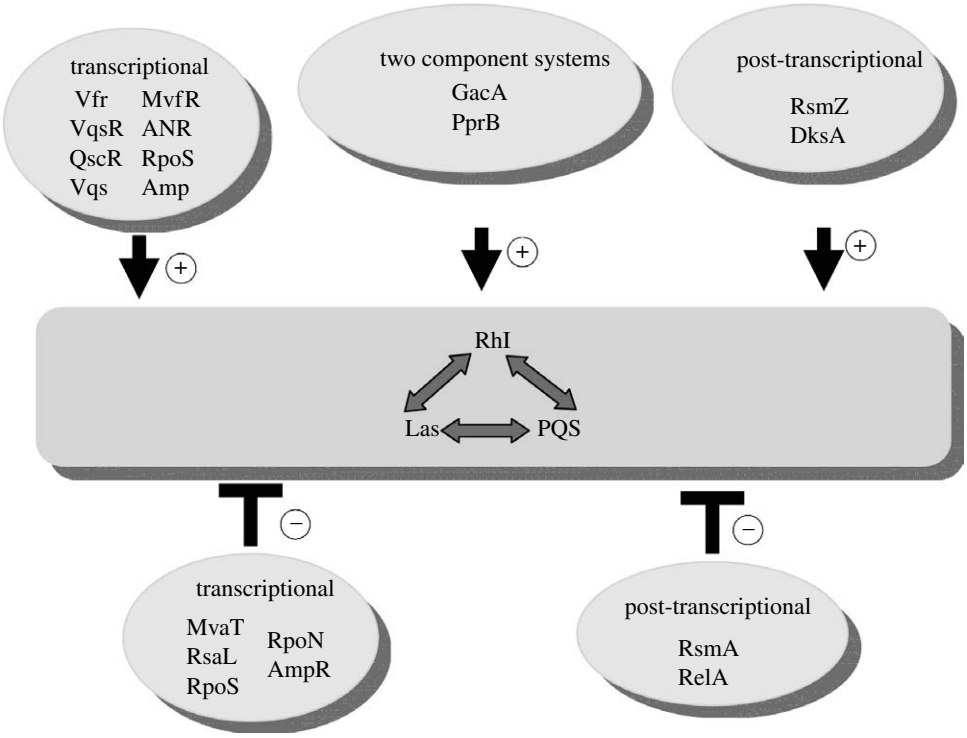

Figure 4. Integration of the *P. aeruginosa* AHL and PQS quorum sensing systems with other regulatory elements operating at the transcriptional and post-transcriptional levels to facilitate environmental adaptation at both population and single cell levels.

phosphorylation, and (probably) degradation in *Salmonella typhimurium* (Taga *et al.* 2001, 2003) and *E. coli* (Wang *et al.* 2005*a*; Xavier & Bassler 2005), i.e. the *lsr* system, comprising an ABC transporter, an AI-2 kinase, and putative enzymes for the subsequent conversion of phosphorylated AI-2 (where AI-2, in this case, is in the form of (2*R*,4*S*)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran (*R*-THMF)) which, after binding to the periplasmic binding protein LsrB, is transported into the cytoplasm where ATP-dependent phosphorylation takes place (Taga *et al.* 2003). It is thought that AI-2 phosphorylation produces the active intracellular signal which mediates quorum sensing-dependent gene regulation. However, alternative views have been presented. For instance, the *lsr* system may simply act to retrieve and degrade a diffusible metabolite (Winzer *et al.* 2003; figure 4).

In many instances, a role for AI-2 in intra- and interspecies signalling has been proposed (Bassler 1999; Schauder *et al.* 2001; Federle & Bassler 2003; Henke & Bassler 2004*c*; Kaper & Sperandio 2005; Xavier & Bassler 2005). However, in the vast majority of these studies, only indirect or incomplete evidence for AI-2-dependent signalling has been provided (Vendeville *et al.* 2005) as these analyses have been complicated by the fact that LuxS also plays a metabolic role in the activated methyl cycle (AMC; figure 5). This cycle is responsible for the generation of the major methyl donor *S*-adenosyl-L-methionine (SAM) and the recycling of methionine from the toxic *S*-adenosyl-L-homocysteine (SAH), which is formed as a product of SAM-dependent methylation reactions (Winzer *et al.* 2003). LuxS takes part in this cycle by salvaging the homocysteine moiety from the cycle intermediate SRH, forming DPD as a by-product (Duerre & Walker 1977; Schauder *et al.* 2001; Zhu *et al.* 2003*b*). Two versions of the AMC exist (figure 5; Walker & Duerre 1975; Winzer *et al.* 2002*a*, 2003). Eukaryotes, archaebacteria, but also many eubacteria use the enzyme SAH hydrolase to convert toxic SAH into homocysteine and adenosine (thus they do not produce DPD/AI-2). Other eubacteria generate homocysteine in the combined reactions of Pfs (methylthioadenosine/*S*-adenosyl-L-homocysteine nucleosidase: converts SAH to SRH and adenine) and LuxS. Presently, there is only one bacterium known to possess both variants of the AMC, *Bifidobacterium longum* (Winzer *et al.* 2003).

The fact that the vast majority of organisms contain a complete AMC suggests that its functions are important for metabolism and thus overall fitness (Winzer *et al.* 2002*a*, 2003). However, it has been argued that the Pfs enzyme is sufficient for the detoxification of SAH, and that bacteria use the Pfs-LuxS variant of the AMC because it allows them to generate the AI-2 signal (Xavier & Bassler 2003). Indeed, an *E. coli pfs* mutant shows a severe growth defect (Cadieux *et al.* 2002), even in complex media, whereas this has not been reported for *luxS* mutants in the same or other genetic backgrounds. On the other hand, *pfs* and *luxS* genes, in agreement with their role in methionine recycling, are often located next to genes involved in sulphur metabolism, in particular those linked to de novo synthesis of cysteine and methionine (Winzer *et al.* 2003). The only currently known biological role of DPD is that of being a direct AI-2 precursor. Indeed, it is intriguing that formation of this molecule is so closely coupled with the metabolic flux through the AMC (one molecule of DPD is formed per SAM-dependent methylation event), making it an ideal signal for metabolic activity and cell population density (Beeston & Surette 2002; Winzer *et al.* 2003; Xavier & Bassler 2003). However, it is also possible that in many

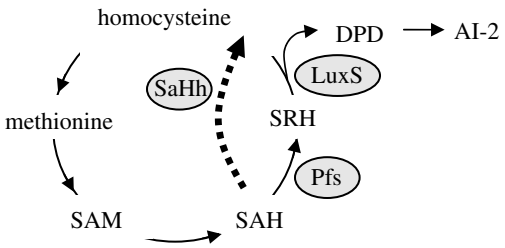

Figure 5. The activated methyl cycle (AMC) drives the formation of methionine and its subsequent conversion to *S*-adenosylmethionine (SAM) which is primarily used for the methylation of DNA, RNA, proteins and certain metabolites. Donation of the SAM methyl group leads to formation of the toxic metabolite *S*-adenosylhomocysteine (SAH). SAH is removed by one of two mechanisms involving either one (SAHh) or two enzymes (Pfs and LuxS) to generate homocysteine and complete the AMC cycle. The Pfs/LuxS pathway also leads to the generation of DPD which spontaneously cyclizes to generate the furanones which constitute AI-2.

bacteria the generation of DPD is not required for signalling but metabolic purposes.

Various phenotypes have been linked to the inactivation of *luxS* in different bacteria, but often it has not been established whether these were caused by a lack of AI-2-dependent signalling or the metabolic perturbations associated with the disruption of the AMC (Winzer *et al.* 2002*a*; Vendeville *et al.* 2005). However, it is clear that *luxS* inactivation in several pathogens affects functions important for virulence such as production of exoenzymes and toxins, motility, and biofilm formation. Accordingly, some *luxS* mutants were shown to be attenuated (Winzer *et al.* 2002*c*; Kim *et al.* 2003; Stroeher *et al.* 2003; Joyce *et al.* 2004; Brandl *et al.* 2005), whereas others showed increased virulence (Daines *et al.* 2005). Significantly, there is mounting evidence to suggest that at least some of these phenotypes are AI-2-independent. For instance, *luxS* mutants of several species were reported to be impaired in their ability to form mono or mixed-species biofilms. These include *Porphyromonas gingivalis*, *Streptococcus gordonii*, *Streptococcus mutans*, *Streptococcus enterica* ssp., *S. epidermidis* and *Klebsiella pneumoniae* (see Vendeville *et al.* 2005 for a summary). The loss of AI-2 signalling was thought to be responsible for the observed changes, a conclusion based on indirect evidence such as addition of AI-2 containing spent culture supernatants or co-culture of wild type and mutant. Recently, however, De Keersmaecker *et al.* (2005) demonstrated that AI-2 derived from synthetic DPD could not restore biofilm formation by a *S. typhimurium luxS* mutant, whereas introduction of *luxS* under control of its own promoter complemented the defect. Furthermore, *Lactobacillus reuteri luxS* mutants continued to produce biofilms of increased thickness even when exposed to AI-2 derived from cell extracts (Tannock *et al.* 2005). Similarly, addition of *in vitro* synthesized AI-2 failed to restore type III secretion and motility defects in enterohaemorrhagic *E.coli* (Sperandio *et al.* 2003) phenotypes previously attributed to AI-2-based quorum sensing (Sperandio *et al.* 1999, 2001, 2002). Addition of synthetic AI-2 also failed to induce significant changes in the *N. meningitidis* proteome (Schauder *et al.* 2005), although *luxS* mutants had previously been shown to be attenuated (Winzer *et al.* 2002*c*). Growth defects observed for *S. aureus* in a sulphur-limited defined medium were also not caused by a lack of AI-2 or any other *luxS*-dependent diffusible factors (Doherty *et al.* 2006).

Taken together, these reports suggest that at least some *luxS*-dependent phenotypes are of intracellular nature. To disentangle AI-2 signalling and metabolic effects of *luxS* inactivation remains a major challenge of the field. Even for well-understood model organisms such as *E. coli* a systematic analysis of the problem has not yet been undertaken. On the contrary, two recent LuxS-related publications concerning this organism illustrate the current division of the field: a recent expression profiling study by Wang *et al.* (2005*b*) concludes that under the investigated conditions the obtained 'data are consistent with the function of LuxS as an important metabolic enzyme but appear not to support the role of AI-2 as a true signal molecule for *E. coli* W3110', whereas Barrios *et al.* (2006) proposed that in *E. coli* MG1655 'AI-2 stimulates biofilm formation and alters its architecture by stimulating flagellar motion and motility'.

One of the main benefits of the current LuxS/AI-2 debate lies in the increasing awareness that terms such as 'communication' or 'signal molecule' have often been used uncritically and sometimes out of context, particularly in recent years where the field of 'social' microbial behaviour has gained much in popularity (Winzer *et al.* 2002*b*; Keller & Surrete 2006).

6. CONCLUDING REMARKS

The widespread capacity of bacterial populations to co-ordinate their behaviour through cell-to-cell communication is now well established. There is a significant body of published work defining the molecular mechanisms by which bacterial cells communicate. However, most bacterial species do not live in isolation and consequently it is perhaps not surprising that quorum sensing signal molecules impact both on other microbes and higher organisms (plants and animals). For example, 3-oxo-C12-HSL produced by *P. aeruginosa* has a wide spectrum of biological activities. It inhibits both growth and *agr*-mediated quorum sensing in *S. aureus* (Qazi *et al.* 2006), filamentation in *Candida albicans* (Hogan *et al.* 2004), has immune modulatory activity (Telford *et al.* 1998; Chhabra *et al.* 2003) and elicits both pro- and anti-inflammatory responses depending on the concentration and model used (Smith *et al.* 2002; Pritchard *et al.* 2005). 3-oxo-C12-HSL also influences smooth muscle contraction in blood vessels (Lawrence *et al.* 1999) and exerts a marked brady-cardia in live conscious rats (Gardiner *et al.* 2001). For *P. aeruginosa*, an opportunistic pathogen, 3-oxo-C12-HSL not only appears to function as a quorum sensing signal molecule controlling expression of key virulence determinants but also as a means to gain a competitive survival advantage in the presence of other organisms occupying the same ecological niche. Consequently, quorum sensing represents an excellent target for novel antibacterials (Rasmussen & Givskov

2006) and AHLs offer an interesting structural backbone for the design of immune modulatory and other pharmacological agents.

Apart from continuing research directed at refining the molecular basis of our understanding of quorum sensing and in characterizing new signalling 'languages', future research in this fascinating area will clearly benefit from refined approaches taking into account the established concepts and definitions developed in the fields of ecology and evolution, and also begin to determine how cell-to-cell communication operates within complex bacterial consortia such as that found within the human intestinal tract.

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