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

Paul Williams¹,*, Klaus Winzer¹, Weng C. Chan² and Miguel Cámara¹

¹Institute of Infection, Immunity and Inflammation, Centre for Biomolecular Sciences, School of Molecular Medical Sciences, and ²School of Pharmacy, University of Nottingham, Nottingham NG7 2RD, UK

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.

* Author for correspondence (paul.williams@nottingham.ac.uk).

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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. 2002b). 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. 2002b). 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.

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 streptomyccin 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-acetylhomoserine 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. (1992a,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 1. Structures of some representative quorum sensing signalling molecules. 3-oxo-AHL, N-(3-oxoacyl)homoserine lactone; 3-hydroxy-AHL, N-(3-hydroxyacyl)homoserine lactone and AHL, N-acetylhomoserine 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-hydroxy-methyl-γ-butyrolactone; AI-2, autoinducer-2, furanosyl borate ester form; PQS, Pseudomonas quinolone signal, 2-heptyl-3-hydroxy-4(1H)-quinolone; DSF, ‘diffusible factor’, methyl dodecanedioic acid; PAME, hydroxyl-palmitic acid methyl ester.](http://rstb.royalsocietypublishing.org/Downloaded from http://rstb.royalsocietypublishing.org/ on October 18, 2017)
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 detoxify the molecule (Winzer et al. 2002b).

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; Câmara et al. 2002b; Chhabra et al. 2005) (table 1). Numerous AHL biosensor assays based on lux, gfp or lacZ reporter gene fusions (Bainton et al. 1992a; 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

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.
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,

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.)

<table>
<thead>
<tr>
<th>organism</th>
<th>major AHL(s)</th>
<th>LuxR</th>
<th>LuxI</th>
<th>phenotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aeromonas hydrophila</td>
<td>C4-HSL</td>
<td>AhyR</td>
<td>AhyI</td>
<td>biofilms, exoproteases</td>
</tr>
<tr>
<td>Aeromonas salmonicana</td>
<td>C4-HSL</td>
<td>AsaR</td>
<td>AsaI</td>
<td>exoprotease</td>
</tr>
<tr>
<td>Agrobacterium tumefaciens</td>
<td>3-oxo-C8-HSL</td>
<td>TraR</td>
<td>TraI</td>
<td>plasmid conjugation</td>
</tr>
<tr>
<td>Agrobacterium vitae</td>
<td>C14:1-HSL, C3-oxo-C16:1-HSL</td>
<td>AysR</td>
<td>AysI</td>
<td>virulence</td>
</tr>
<tr>
<td>Burkholderia cenocepacia</td>
<td>C6-HSL, C8-HSL</td>
<td>CepR</td>
<td>CciI</td>
<td>exoenzymes, biofilm formation, swarming motility, siderophore, virulence, exoprotease</td>
</tr>
<tr>
<td>Burkholderia pseudonallei</td>
<td>C8-HSL, C10-HSL, 3-hydroxy-C8-HSL, 3-hydroxy-C10-HSL, 3-hydroxy-C14-HSL</td>
<td>PmII1, PmII2, PmII3</td>
<td>Virulence</td>
<td></td>
</tr>
<tr>
<td>Burkholderia mallei</td>
<td>C8-HSL, C10-HSL</td>
<td>BmaR1, BmaR3, BmaR4, BmaR5</td>
<td>BmaI1, BmaI3</td>
<td>Virulence</td>
</tr>
<tr>
<td>Chromobacterium violaceum</td>
<td>C6-HSL</td>
<td>CviR</td>
<td>CviI</td>
<td>exoenzymes, cyanide, pigment, carbapenem, exoenzymes, virulence, exopolysaccharide, swimming motility, virulence</td>
</tr>
<tr>
<td>Erwinia carotovora ssp. carotovora</td>
<td>3-oxo-C6-HSL</td>
<td>ExpR/CarR</td>
<td>Carl</td>
<td>(ExpI) carbapenem, exoenzymes, virulence, exopolysaccharide, swimming motility, virulence</td>
</tr>
<tr>
<td>Pantoea (Erwinia) stewartii</td>
<td>C4-HSL; 3-oxo-C12-HSL</td>
<td>LasR, RhlR, QscR, VqsR</td>
<td>LasI, RhlI</td>
<td>exoenzymes, secretion, HCN, biofilms</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>C6-HSL</td>
<td>PhzR, CsaR</td>
<td>PhzI</td>
<td>CsaI</td>
</tr>
<tr>
<td>Pseudomonas putida</td>
<td>3-oxo-C10-HSL, 3-oxo-C12-HSL</td>
<td>PpuR</td>
<td>PpuI</td>
<td>phenazine-1-carboxamide, exopolysaccharide, swimming motility, virulence</td>
</tr>
<tr>
<td>Pseudomonas chlororaphis</td>
<td>C6-HSL</td>
<td>PhzR</td>
<td>PhzI</td>
<td>exoenzymes, cyanide, pigment, carbapenem, exoenzymes, virulence, exopolysaccharide, swimming motility, virulence</td>
</tr>
<tr>
<td>Pseudomonas syringae</td>
<td>3-oxo-C6-HSL</td>
<td>AhlR</td>
<td>AhlI</td>
<td>exopolysaccharide, swimming motility, virulence</td>
</tr>
<tr>
<td>Rhizobium leguminosarum bv viciae</td>
<td>7-cis-C14-HSL/C6-HSL, C7-HSL/C8-HSL, 3-oxo-C6-HSL, 3-hydroxy-C8-HSL</td>
<td>CinR, CinI, Rhl, RhlI</td>
<td>CinR, Rhl, RhlI</td>
<td>root nodulation/symbiosis, plasmid transfer, growth inhibition, stationary phase adaptation</td>
</tr>
<tr>
<td>Rhodobacter sphaeroides</td>
<td>7-cis-C14-HSL</td>
<td>CerR</td>
<td>CerI</td>
<td>aggregation</td>
</tr>
<tr>
<td>Serratia spp. ATCC 39006</td>
<td>C4-HSL</td>
<td>SmaR</td>
<td>SmaI</td>
<td>antibiotic, pigment, exoenzymes, swimming motility, exoprotease, biofilm development, biosurfactant</td>
</tr>
<tr>
<td>Serratia liquefaciens MG1</td>
<td>C4-HSL</td>
<td>SwrR</td>
<td>SwrI</td>
<td>sliding motility, biosurfactant, pigment, nuclease, transposition frequency</td>
</tr>
<tr>
<td>Serratia marcescens SS-1</td>
<td>C6-HSL, 3-oxo-C6-HSL</td>
<td>SpnR</td>
<td>SpnI</td>
<td>sliding motility, biosurfactant, pigment, nuclease, transposition frequency</td>
</tr>
<tr>
<td>Serratia proteamaculans B5a</td>
<td>3-oxo-C6-HSL</td>
<td>SprR</td>
<td>SprI</td>
<td>exoenzymes</td>
</tr>
<tr>
<td>Vibrio fisheri</td>
<td>3-oxo-C6-HSL</td>
<td>LuxR</td>
<td>LuxI</td>
<td>bioluminescence, swimming and swarming motility</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>C6-HSL, 3-oxo-C6-HSL, 3-oxo-C10-HSL, 3-oxo-C12-HSL, 3-oxo-C14-HSL, 3-oxo-C16:1-HSL, C16:1-HSL, C18-HSL</td>
<td>YenR, YenI</td>
<td>YenI</td>
<td>motility, aggregation</td>
</tr>
<tr>
<td>Yersinia pseudotuberculosis</td>
<td>C6-HSL, 3-oxo-C6-HSL, C8-HSL</td>
<td>YpsR, YtbR</td>
<td>YpsI, YtbI</td>
<td>motility, aggregation</td>
</tr>
</tbody>
</table>
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-acetyl, 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-acetyl, 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, Versinia 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 located on plasmids such as the Agrobacterium Ti plasmid (Zhang et al. 1993) and Rhizobium Symbiotic plasmids (Wisniewsky-Dwe & 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 (TnTIR) 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 (More 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, EsI 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 EsI 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 (ValnI 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 (Câmara et al. 2002a; 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; Winston 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; Winston 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 las rhl II 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 disassociate, 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). Furthermore, 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; Diggel 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 (Diggel et al. 2002), the anaerobic regulator ANR (Pessi & Haas 2000), the AraC-type regulator VqsM (Dong et al. 2005a) and two members of the two component signal transduction response regulator family, namely GacA (Reimmann et al. 1997) and PrpB (Dong et al. 2005b), 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. 2005a), 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(1H)-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; Diggel 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 (Diggel 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 (Arendenkerk 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 macroring 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, nisABCIPRTKFEFG. It is a self-inducible system involving the two-component regulatory system NisK–NisR which controls the expression of proteins involved in nisin biosynthesis (NisABC) 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 Cα of the indole side-chain, as well as stereospecific intramolecular (indole C2→N3) 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 farnesyI; 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

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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), tss (TSST-1), sps 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 nine amino acid residues.

### 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 (Schauer et al. 2001; Xavier & Bassler 2003) is an iron-containing enzyme (Zhu et al. 2003a) 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, spirochaetes, firmicutes, and actinobacteria, 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 V. cholerae, where it is involved in the regulation of bioluminescence and virulence-associated traits, respectively (Miller et al. 2002; Xavier & Bassler 2003; Henke & Bassler 2004a,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 2004a).

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,

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phosphorylation, and (probably) degradation in *Salmonella typhimurium* (*Taga* et al. 2001, 2003) and *E. coli* (*Wang et al. 2005a; 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 (2R,4S)-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 2004c; 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-1-methionine (SAM) and the recycling of methionine from the toxic *S*-adenosyl-1-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*). Two versions of the AMC exist (*figure 5*; *Walker & Duerre 1975; Winzer et al. 2002a, 2003*). Eukaryotes, archaeabacteria, 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-1-homocysteine nucleosidase: converts SAH to SRH and adenosine) 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. 2002a, 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
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. 2002a; 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. 2002a; Kim et al. 2003; Stroecher 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 spp., 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 with the wild-type strain (Telford et al. 2001). 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. 2002c). 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. (2005b) 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. 2002b; 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; Prritchard 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 2002).
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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