Review

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Microbial dinner-party conversations: the role of LuxS in interspecies communication

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Bacteria have a tendency to be gregarious by nature. Whether on abiotic surfaces in the environment or on the mucosal surfaces of humans, bacteria accumulate in complex multi-species communities. In these dynamic accretions, bacteria can be densely packed and often depend on each other for the provision of metabolic substrates. Under these circumstances, it will be advantageous for bacteria to be able to detect the presence of their neighbours, to communicate with them and to co-ordinate various physiological activities. Such cell–cell sensing and communication systems can be established through the release and detection of chemical signalling molecules. While originally considered a feature characteristic of eukaryotes, the exchange of chemical signals has now been demonstrated in many bacterial species and ecosystems. Indeed, it has even been suggested that assemblages of bacterial species can be considered as proto-multicellular organisms, whereby biological processes are controlled for the benefit of the entire community. Regardless of the extent to which bacterial communication represents a step on the road to multicellularity, it is becoming increasingly apparent that the signalling systems devised by bacteria are essential for successful relationships with other bacteria and with eukaryotic hosts.

The chattering classes

Population density is used by bacteria as a cue for the regulation of diverse cellular functions. Quorum (density-dependent) sensing involves the synthesis of small signalling molecules that accumulate in the local environment. As the bacterial population density increases, the concentration of signalling molecules also increases until a threshold, or quorum, is reached, triggering the expression of target genes. In Gram-negative bacteria, the best-described and most widespread signalling molecule is acyl homoserine lactone (AHL) (Fuqua et al., 2001). In the archetypal quorum-sensing system of the marine symbiotic bacterium Vibrio fischeri, AHL is synthesized by LuxI protein. At a threshold concentration, the membrane-diffusible AHL interacts with and activates the intracellular LuxR transcriptional activator resulting, in the case of V. fischeri, in expression of genes required for bioluminescence (Fig. 1a). LuxI/R homologues have now been described in a large group of phylogenetically diverse bacteria including numerous plant and animal pathogens and, in many instances, two or more LuxI/R systems may function in a single species. AHL-based quorum sensing is known to regulate a range of cellular functions including bioluminescence, motility, production of secondary metabolites, expression of virulence factors and plasmid conjugal transfer (Fuqua et al., 2001; Miller & Bassler, 2001; Schauder & Bassler, 2001). Signalling through AHLs has also been shown to be important for biofilm formation in some organisms (Davies et al., 1998), although other factors such as hydrodynamics appear to be at least as important (Kjelleberg & Molin, 2002; Purevdorj et al., 2002). The AHLs produced by LuxI enzymic activity are system specific, and AHLs have been described with N-linked acyl side-chain lengths varying between 4 and 14 carbons, with various substitutions at the C3 position of the side chain. The LuxR proteins are exquisitely sensitive to their cognate AHL; however, they may respond to non-cognate AHLs, albeit at higher concentrations of the AHL (Fuqua et al., 2001; Parsek & Greenberg, 2000).

LuxI/R systems have not yet been described in Gram-positive bacteria. Instead, many genera possess quorum-sensing systems based on modified or unmodified peptides. Not only is the language of communication different, but the means by which Gram-positive bacteria detect and act upon a threshold level of quorum-sensing signal may also differ from the LuxI/R system (Dunny & Leonard, 1997). One of the best-described systems is regulation of competence for genetic transformation in Streptococcus pneumoniae (Claverys et al., 2000; Morrison & Lee, 2000). The comCDE locus, encoding competence-stimulating peptide (CSP), histidine kinase receptor and cognate response regulator, is transcribed at low levels during growth. The 41 amino acid product of comC is secreted from cells, as an active mature
heptadecapeptide, by a dedicated ATP-binding-cassette (ABC) transporter that is encoded by the unlinked comAB locus. The threshold level of CSP is detected at the cell surface by the ComD histidine kinase, which subsequently phosphorylates the response regulator ComE. Thus activated, ComE switches on the genes required for DNA uptake and processing (Fig. 1b). Competence in other streptococci, and the expression of antimicrobial peptides by a range of Gram-positive bacterial genera, is regulated in a similar fashion. In Bacillus subtilis, sporulation and competence are regulated by a complex network of phosphorylation and dephosphorylation events influenced by numerous peptide signals (Grossman, 1995). Some peptide signals are detected at the cell surface by LuxN and LuxQ, which subsequently converge in a common pathway to regulate bioluminescence genes in response to cell density (Fig. 1c) (Freeman et al., 1999a, b).

**Talking the talk and walking the walk**

Following its discovery in *V. harveyi*, in silico analyses revealed homologues of *luxS* in a wide range of organisms, including a number of important human pathogens. Most of these species were also able to produce functional AI-2, as measured by induction of bioluminescence in the *V. harveyi* reporter strain. As these organisms are not bioluminescent, nor likely to colonize the light organs of marine animals, the role of AI-2 must extend beyond light induction. Intriguingly, it appears that different organisms co-opt LuxS-based signalling for different purposes. Utilizing microarrays, Sperandio et al. (2001) found that a luxS null mutant of enterohaemorrhagic *E. coli* (EHEC) demonstrated differential expression of over 400 genes; remarkably, around 10 % of the total number of genes on the array. LuxS-downregulated genes included those involved in cell division, along with ribosomal and tRNA genes. Upregulated genes included several virulence factors such as genes involved in flagella biogenesis, motility and chemotaxis. The same group has also
shown that the type-III secretion system, along with intimin and its translocated receptor, are regulated by LuxS in both EHEC and enteropathogenic E. coli (Sperandio et al., 1999). Similarly, DiLisa et al. (2001) found 242 genes (approx. 5% of the genome) of E. coli that responded to the presence of AI-2, delivered in conditioned medium. Many of these genes are related to virulence or to processes such as cell division, morphogenesis and cell-surface architecture. A role for LuxS signalling as both a global regulator and as important for expression of virulence traits is thus emerging. In Porphyromonas gingivalis, AI-2 can control expression of genes involved in haemin uptake and in haemagglutination (Burgess et al., 2002; Chung et al., 2001). This periodontal pathogen has a requirement for haemin as a source of iron. Furthermore, haemin levels can also regulate expression of virulence properties by the organism. AI-2 signal is also required for formation of a mixed species biofilm with the oral commensal Streptococcus gordonii (McNab et al., 2003). Consistent with its role as a species-specific signal, AI-2 from S. gordonii can complement a luxS mutation in P. gingivalis. Furthermore, P. gingivalis can also respond to AI-2 signal from another oral pathogen, Actinobacillus actinomycetemcomitans (Fong et al., 2001). In A. actinomycetemcomitans itself, production of leukotoxin, an important virulence factor, and iron-uptake proteins is regulated in response to LuxS (Fong et al., 2001). The luxS gene is also present in a range of pathogenic Gram-positive bacteria including Streptococcus mutans, Streptococcus pyogenes and Clostridium perfringens (Lyon et al., 2001; Ohtani et al., 2002; Wen & Burne, 2002). In the latter two instances, LuxS may be associated with virulence through the regulation of expression or activity of virulence factors, including haemolytic activity and SpeB-extracellular cysteine protease, and secreted toxins, respectively.

Nonetheless, despite the frequently observed connection between LuxS and pathogenicity, results of studies testing luxS mutants in models of disease have been disappointing. P. gingivalis luxS mutants were found not to be attenuated in a murine lesion model of infection nor to be deficient in intracellular invasion of host epithelial cells (Burgess et al., 2002; Chung et al., 2001). Similarly, while Shigella flexneri LuxS modulates expression of virF, a transcription factor necessary for the expression of invasion loci, mutants deficient in AI-2 were fully virulent in the in vivo Sereny test (Day & Maurelli, 2001). An exception to this was the demonstration that luxS mutants of Neisseria meningitidis are defective for bacteremia in a rat model (Winzer et al., 2002c). Complementation of the mutation by luxS restored virulence to wild-type levels. The influence of LuxS on pathogenicity may, therefore, be subtle and interconnected with other regulatory pathways such that a loss of LuxS is only manifest during certain stages of disease.

Talking with their mouths full

The LuxS system was originally discovered as a second quorum-sensing circuit controlling bioluminescence in V. harveyi. Moreover, in V. harveyi, the LuxQ sensor kinase protein that detects AI-2 is upstream of LuxU, an integrator protein that is common to the AI-1 circuit (Freeman & Bassler, 1999a, b). These elegant studies from Bassler’s group firmly established V. harveyi LuxS/AI-2 as a quorum-sensing mechanism in the tradition of the LuxR/LuxI system. The discovery of luxS homologues and AI-2 activity in other non-bioluminescent bacteria led to the understandable assumption that this also constituted LuxS-dependent quorum sensing. However, in their enthusiasm to explore this emerging field of bacterial gene regulation, investigators (such as ourselves!) rarely demonstrated formally that LuxS-based signalling was strictly cell-density dependent. While inimetal to the issue of what LuxS controls, the notion that the primary role of LuxS-based signalling may not be in cell-to-cell communication has been proposed by Winzer et al. (2002a). Based on the premise that a true signalling molecule generates responses beyond a physiological mechanism required to metabolize or detoxify the signal, LuxS can be viewed in terms of metabolic regulation. LuxS is an enzyme that converts S-ribosylhomocysteine (RH) to 4,5-dihydroxy-2,3-pentanedione (DPD) and homocysteine (Fig. 2) (Schauer et al., 2001). In the presence of boron, DPD will form a furanosyl borate diester that has AI-2 activity (Chen et al., 2002). Upstream of the LuxS-catalysed step, RH is produced by the action of the Pfs enzyme (encoding a 5′-methylthioadenosine-5′-adenosylhomocysteine nucleosidase) on S-adenosylhomocysteine (SAH). SAH is formed from S-adenosylmethionine (SAM), a major methyl donor, following methylation of a number of substrates including DNA, RNA and proteins. LuxS is thus a component of an important SAH-degradation pathway that reduces feedback inhibition of SAM-dependent methylation and will also allow the scavenging of SAH constituents (Fig. 2) (Winzer et al., 2002b). The subsequent accumulation of AI-2 may be damaging to the cellular DNA and, hence, AI-2 is extruded from the cell. As more methylation reactions are required

![Fig. 2. Simplified diagram of the active methyl cycle. The conversion of S-ribosylhomocysteine (RH) to homocysteine and 4,5-dihydroxy-2,3-pentanedione (DPD) is catalysed by LuxS. The conversion of DPD to AI-2 or to 4-hydroxy-5-methyl-3(2H)-furane (not shown) is thought to occur spontaneously. See text for more detail.](http://jnm.sgmjournals.org)
when cells are growing rapidly, the increase in extracellular AI-2 signal during exponential phase may simply reflect the activity of the methyl cycle rather than density dependence. Furthermore, AI-2 may be taken up and degraded by the cells later in order to recapture constituent molecules. A number of experimental observations can be interpreted as supporting this hypothesis. For example, in *P. gingivalis*, *pfs* and luxS form an operon (Chung et al., 2001). Moreover, in the absence of glucose, AI-2 activity in *S. Typhimurium* is reduced (Surette & Bassler, 1999). This may be the result of AI-2 degradation in order to provide the cells with an additional metabolic substrate, the explanation favoured by Winzer et al. (2002a). As noted by Surette & Bassler (1999), however, the loss of AI-2 activity could equally well result from termination of production. Furthermore, these authors also reported that *S. Typhimurium* growing on several other carbon sources (acetate, glycerol, citrate and serine), and thus presumably utilizing the activated methyl cycle, does not produce AI-2 signal activity. More recently, Beeston & Surette (2002) reported that, in *S. Typhimurium*, expression of luxS is constitutive and that neither luxS nor pfs expression is regulated by AI-2. They concluded that AI-2 production is regulated at the level of LuxS substrate availability and that AI-2 signalling is a reflection of the metabolic state of the cell. Certainly, therefore, a persuasive case can be made for LuxS as having been originally designed as a component of a metabolic/detoxification pathway. Nevertheless, the large number and range of genes that appear to be controlled by LuxS would suggest relevance beyond the activated methyl cycle.

**Noise annoys**

Another question that remains to be answered relates to interference, or background ‘noise’, that would be inherent with a universally detected signal. On the basis that culture supernatant from many diverse bacteria can induce bioluminescence in *V. harveyi*, AI-2 can certainly be considered species non-specific. What is less clear, however, is whether all bacteria sense and respond equally to heterologous AI-2. Given that large numbers of genes can be controlled by AI-2 signalling, it would seem that individual bacteria in densely packed communities could be overwhelmed by AI-2 noise from other species. Is it possible, therefore, that AI-2 signalling could have a degree of specificity overlaid on its interspecies activity? One way to accomplish this would be for bacteria to produce unique modifications to the basic AI-2 structure that, while recognizable as AI-2, would also impart a degree of specificity. Some support for this concept is provided by the finding that culture supernatant from *P. gingivalis* and *S. gordonii* is about one log less active in *V. harveyi* bioluminescence than is the homologous signal (Chung et al., 2001; McNab et al., 2003). Specificity could also occur at the level of signal transduction. In *V. harveyi*, two nutritional regulators, CRP (CAMP receptor protein) and MetR (a LysR homologue), bind to bioluminescence gene promoters (Chatterjee et al., 2002). However, CRP is an activator of gene expression, whereas MetR represses gene activity. Differential expression of regulatory proteins could, therefore, be one means of integrating multiple environmental and cell-density signals and ensuring a controlled response to AI-2. Resolution of this issue awaits the elucidation of the structure of AI-2, and of the molecular circuitry of AI-2 detection, in a wider range of organisms.

**The last word**

While some aspects of bacterial communication are now well understood, much remains to be learned about the roles of the players involved in the LuxS drama. As bacteria are constantly striving to enhance their metabolic state (with subsequent increase in cell density), signalling and metabolism are intricately interconnected. Hence, it will not be a simple task to distinguish regulation of gene expression as a function of signalling activity per se from differential expression of genes induced by the metabolic state of the cell. In either event, it is a relatively common occurrence for bacteria to exploit one protein or one pathway for multiple purposes. Perhaps the more important issue is not how to define LuxS but, rather, what can LuxS-dependent systems tell us about the pathophysiology of medically relevant bacteria?

**References**


