How bacteria talk to each other: regulation of gene expression by quorum sensing

Bonnie L Bassler

Quorum sensing, or the control of gene expression in response to cell density, is used by both Gram-negative and Gram-positive bacteria to regulate a variety of physiological functions. In all cases, quorum sensing involves the production and detection of extracellular signalling molecules called autoinducers. While universal signalling themes exist, variations in the design of the extracellular signals, the signal detection apparatuses, and the biochemical mechanisms of signal relay have allowed quorum sensing systems to be exquisitely adapted for their varied uses. Recent studies show that quorum sensing modulates both intra- and inter-species cell–cell communication, and it plays a major role in enabling bacteria to architect complex community structures.

Addresses
Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544-1014, USA; e-mail: bbassler@molbiol.princeton.edu

Current Opinion in Microbiology 1999, **2**:582–587

1369-5274/99/$ – see front matter

Abbreviations

ABC	ATP-binding cassette
CSF	competence and sporulation factor
HSL	homoserine lactone

Introduction

Research in bacterial quorum sensing began with studies of the density-dependent expression of bioluminescence in the marine symbiotic bacterium *Vibrio fischeri* and its free-living relative *Vibrio harveyi* [1,2]. Both species produce and respond to secreted acylated-homoserine lactone (HSL) signalling molecules called autoinducers that accumulate in the external environment as the cells grow [3,4]. When the concentration of autoinducer exceeds a threshold level, a signal transduction cascade is initiated that leads to the production of luciferase. The crucial findings of Engebrecht and Silverman [5–7] laid the foundation for all subsequent studies of quorum sensing in Gram-negative bacteria. They identified, cloned, and analysed the genes encoding the luciferase enzyme complex and the genes responsible for its density-dependent regulation from *V. fischeri*. They showed that light production in *V. fischeri* is controlled by two regulatory proteins named LuxI and LuxR. LuxI is the autoinducer synthase that is responsible for the synthesis of the acyl-HSL autoinducer. LuxR is a transcriptional activator protein that, when bound to autoinducer, promotes transcription of the luciferase structural operon *luxCDABE* [5–7]. These observations first explained how gene expression could be coupled to cell-population density.

This review focuses on recent advances in how bacteria regulate gene expression in response to cell density. Specifically, this review highlights major differences and similarities in the mechanisms employed for quorum sensing in Gram-negative and Gram-positive bacteria. Recent findings that demonstrate how sophisticated signalling networks are employed in these cell–cell communication systems are discussed.

Gram-negative bacterial communication: the LuxI/LuxR language

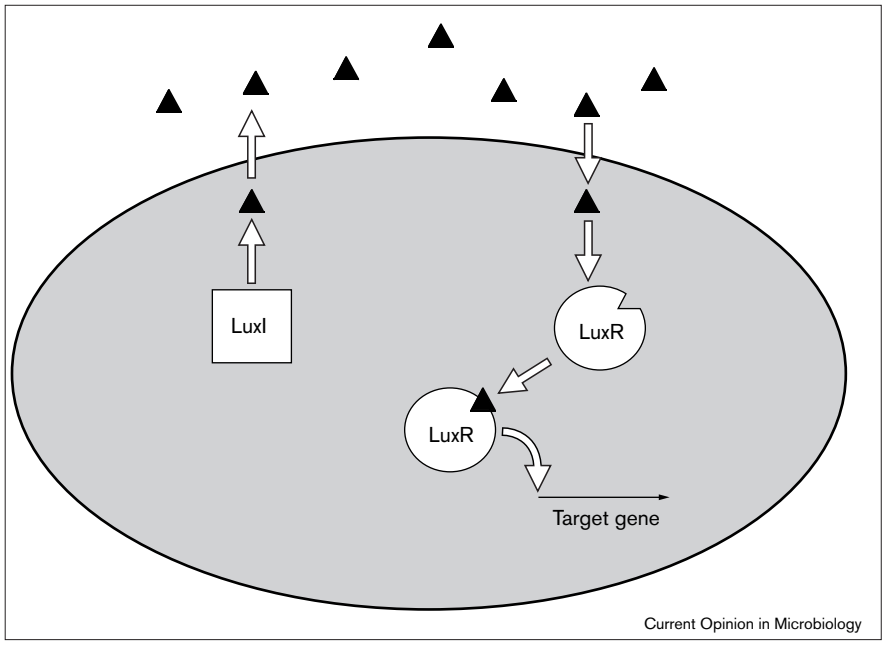
The simple signal-response mechanism described by Engebrecht and Silverman has now been shown to be employed by over 30 species of Gram-negative bacteria for the control of different cell-density-dependent functions [8,9]. These systems all have in common the use of an HSL autoinducer whose synthesis is dependent on a *luxI* homologue, as well as a *luxR* homologue encoding a transcriptional activator protein that is responsible for detection of the cognate HSL and induction of expression of the appropriate output (Figure 1).

Recently, it has become clear that additional complexity exists in many of these LuxI/LuxR systems. For example, in the opportunistic pathogen *Pseudomonas aeruginosa*, two LuxI/R pairs exist (LasI/R, RhlI/R) and function in tandem to control the expression of virulence factors [10–15]. In *Ralstonia solanacearum*, a phytopathogenic bacterium, quorum sensing controls the production of virulence factors including plant cell-wall-degrading enzymes. Expression of the *R. solanacearum* LuxI/LuxR-like autoinduction system (SolI/SolR) is regulated by a LysR-like transcriptional regulator called PhcA that responds to 3-hydroxy-palmitic acid methyl ester. The SolI/SolR system is also controlled by RpoS, the stationary phase sigma factor [16,17,18•]. In *Agrobacterium tumefaciens*, the plant pathogen responsible for crown gall tumours, quorum-sensing outputs are responsive to both bacterial and host signals. In this system, plant opine hormones interact with either the bacterial protein OccR or AccR to regulate the expression of the *luxR* homologue *traR* [19,20•,21]. Many other examples exist in which the backbone of the quorum sensing mechanism is a LuxI/LuxR signal-response circuit, upon which further levels of regulation have been layered [22–24]. Note that density-dependent gene regulation is important for the control of sporulation in the Gram-negative bacterium *Myxococcus xanthus*. This system is quite different from HSL quorum sensing, and beyond the scope of this review. It is addressed in detail elsewhere [25,26].

Gram-positive bacteria have their own language

There exist a number of processes in Gram-positive bacteria that are responsive to cell population density. Among these are competence for DNA uptake in *Bacillus subtilis*

Figure 1



LuxI/LuxR quorum sensing. In most Gram-negative quorum sensing bacteria, LuxI-like autoinducer synthases (square) are responsible for production of specific HSL autoinducers (triangles). This class of autoinducer freely diffuses across the bacterial membrane. Upon reaching a critical concentration, the autoinducer is bound by its cognate LuxR-like protein (circle), and together the LuxR–HSL autoinducer complex activates transcription of the target gene(s).

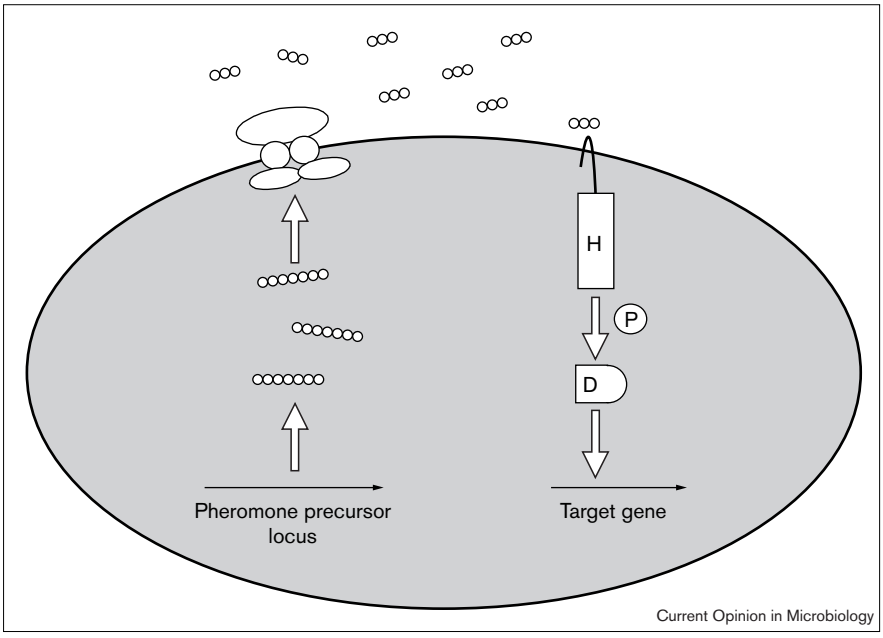
and *Streptococcus pneumoniae*, virulence in *Staphylococcus aureus*, conjugation in *Enterococcus faecalis* and microcin production in *Lactobacillus sake* and *Carnobacterium piscicola*. Gram-positive bacteria do not employ HSLs as signals, nor do they use a LuxI/LuxR signalling circuit. Instead, Gram-positive bacteria secrete processed peptide signalling molecules usually via a dedicated ABC (ATP-binding cassette) exporter protein. The peptide signals are recognised by cognate two-component sensor kinase proteins that interact with cytoplasmic response regulator proteins. The mechanism of signal transduction is a phosphorelay cascade (Figure 2) [27,28].

Similar to Gram-negative LuxI/LuxR signalling, Gram-positive bacteria employ a common signalling substructure, in this case a two-component circuit, with variations in the type and complexity of additional regulatory factors. For example, in *B. subtilis*, two processed peptide signals enable the bacteria to choose between competence for DNA uptake and sporulation. The secretion machinery necessary for export of these two peptides has not been identified. One of the extracellular peptides, ComX, activates the ComP/ComA two-component system to allow the transition to the transformable state. The second peptide signal, CSF (competence and sporulation factor), is imported by an ABC transporter. A low internal concentration of CSF promotes competence development, whereas a high internal concentration of CSF inhibits competence and induces sporulation [29,30•]. In *E. faecalis*, several peptide signals are involved in inducing conjugation between plasmid-containing donor cells and plasmid-less recipient cells. Each peptide is encoded by a different plasmid and specifically promotes the acquisition of that particular plasmid [31–33]. Finally, in staphylococci the synthesis of an untranslated RNA molecule called RNA III is under the control of peptide quorum sensing. The RNA III molecule is the effector of the system and is responsible for both positive and negative regulation of a variety of downstream targets including genes encoding protein A, coagulase, enterotoxins and hemolysins [28,34–36].

Hybrid languages: the quorum sensing systems of *V. harveyi*

The free-living marine luminous bacterium *V. harveyi* possesses two autoinducer-response systems that function in parallel to control the density-dependent expression of the luciferase structural operon *luxCDABE*. This complex quorum sensing circuit has features found in both Gram-negative and Gram-positive bacteria. Like other Gram-negative quorum sensing bacteria, *V. harveyi* produces and responds to an acylated-HSL autoinducer. The second *V. harveyi* autoinducer is of unknown structure, but preliminary evidence indicates that it is not a HSL [37,38]. Recognition and response to the two autoinducers occurs via a two-component signal transduction network reminiscent of quorum sensing systems in Gram-positive bacteria. The two *V. harveyi* autoinducers, AI-1 and AI-2, are recognised by cognate sensor kinase proteins named LuxN and LuxQ, respectively. Additionally, a periplasmic-binding protein called LuxP is hypothesised to interact with LuxQ to recognise AI-2 [37,38]. Sensory information from both systems is transduced by phosphorylation and dephosphorylation to a shared signal integrator protein called LuxU, which subsequently conveys the signal to the response regulator protein LuxO (Figure 3) [39,40,41••,42•]. Interestingly, no LuxI/LuxR homologues function in the *V. harveyi* quorum sensing system. Production of the *V. harveyi* HSL autoinducer AI-1 is dependent on the

Figure 2



Peptide quorum sensing. In most Gram-positive quorum sensing bacteria, dedicated ABC transporters process and export peptide autoinducers (pheromone). Extracellular pheromones are recognised by membrane bound two-component sensor kinase proteins. The sensors autophosphorylate on a conserved histidine residue (H), and subsequently transfer the phosphoryl group to cognate response regulators. Response regulators are phosphorylated on conserved aspartate residues (D). Following phosphorylation, response regulator proteins activate/repress transcription of specific target gene(s). The ABC transporter is depicted as a protein complex of circles and ovals in the bacterial membrane. The precursor peptide and the processed peptide autoinducer are represented as long and short chains of circles, respectively. In the figure, the length of the peptide chains is not meant to signify any particular number of amino acid residues. The P in the circle denotes that phosphorylation is the mechanism of signal transduction from the sensor kinase to the response regulator.

luxL and *luxM* genes [37], and AI-2 synthesis is dependent on the *luxS* gene [43••]. These genes share no homology to the *luxI* family of autoinducer synthases.

Bacterial Esperanto: the LuxS family of autoinducers

Highly conserved *luxS* homologues have now been identified in both Gram-negative and Gram-positive bacterial species including *Escherichia coli*, *Salmonella typhimurium*, *Salmonella typhi*, *Salmonella paratyphi*, *Haemophilus influenzae*, *Helicobacter pylori*, *B. subtilis*, *Borrelia burgdorferi*, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Yersinia pestis*, *Campylobacter jejuni*, *Vibrio cholerae*, *Deinococcus radiodurans*, *Mycobacterium tuberculosis*, *E. faecalis*, *S. pneumoniae*, *Streptococcus pyogenes*, *Streptococcus mutans*, *Staphylococcus aureus*, *Clostridium perfringens*, *Clostridium difficile*, *Shewanella putrefaciens*, *Klebsiella pneumoniae*, and *Pasteurella multocida* [43••]. Most of the species of bacteria possessing a *luxS* gene have been shown to produce AI-2 activity, and *luxS* mutants have been constructed in *V. harveyi*, *E. coli*, *S. typhimurium*, *V. cholerae* and *H. pylori*. In each case, mutation of *luxS* eliminated AI-2 production ([43••]; BL Bassler, unpublished data). Currently, it is not known what functions are controlled by this class of signalling molecule in any bacterium other than *V. harveyi*, although there are a number of pieces of circumstantial evidence indicating that pathogenicity is regulated by AI-2 in *E. coli*, *S. typhimurium*, and *V. cholerae* ([43••,44••,45]; BL Bassler, unpublished data).

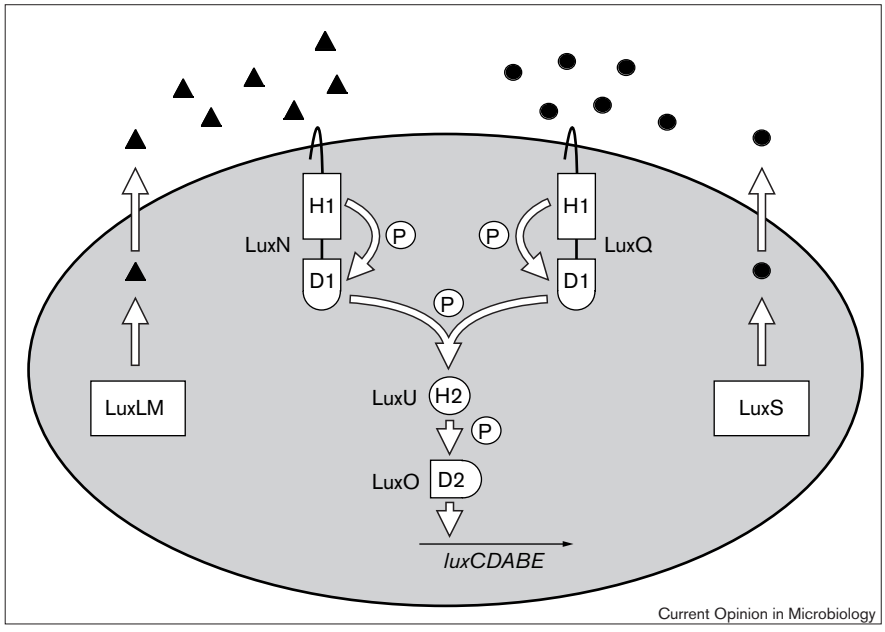
V. harveyi induces *lux* expression in response to the endogenous production of AI-1 and AI-2, but it also responds to AI-2 produced by many of the other bacteria that possess a *luxS* homologue ([45]; BL Bassler, unpublished data). These bacteria include both Gram-negative and Gram-positive species. This result suggests that communication via an AI-2 signal response system could be a common mechanism that bacteria employ for inter-species interaction in natural environments [40,43••,46]. The capacity to respond to both intra- and inter-species signals could allow *V. harveyi* to know not only its own cell density, but also its proportion of the total bacteria in a mixed population. Furthermore, the ability to distinguish self from others could allow *V. harveyi* to differentially control gene expression dependent upon whether it exists in pure culture or in consortium. Other species of bacteria that produce an AI-2 activity could have similar capabilities.

Multilingual bacteria: cell–cell communication in nature

As noted below, there are several fascinating systems currently under study in which the use of intra- and inter-species quorum sensing would be predicted to greatly enhance a particular bacterium's chances of survival, or would allow bacteria to build communities in which specialisation/division of labour would grant the entire community some of the properties and benefits that would otherwise be exclusive to multicellular organisms.

Quorum sensing regulates virulence in many human and plant pathogens. Presumably, in an attempt to avoid alerting the host's immune system to their presence, quorum sensing bacteria delay virulence factor production until cell number is high enough that secretion of virulence factors will result in a productive infection [8,10,16,21,28]. For example, in *S. aureus*, the Agr quorum sensing system regulates the production of virulence factors that enhance

Figure 3



Hybrid quorum sensing in *V. harveyi*. The two quorum sensing circuits of *V. harveyi* are shown. AI-1 (triangles) is a HSL autoinducer, and the structure of AI-2 (circles) has not been determined. Synthesis of AI-1 and AI-2 is dependent on LuxLM and LuxS, respectively. Following the build-up of a critical external concentration of the autoinducers, signalling occurs via a series of phosphorylation/dephosphorylation reactions. The AI-1 and AI-2 detectors, LuxN and LuxQ, respectively, contain both a sensor kinase domain with a conserved histidine (H1) and an attached response regulator domain with a conserved aspartate (D1). Signals from both sensors are channelled to the shared integrator protein LuxU, which is phosphorylated on a histidine residue (H2). Subsequently, the signal is transduced to a conserved aspartate residue (D2) on the response regulator protein LuxO. LuxO–phosphate controls the expression of the luciferase structural operon *luxCDABE*. The phosphoryl flow in the system is H1 to D1 to H2 to D2. The LuxN and LuxQ sensors also possess phosphatase activity, which is responsible for dephosphorylation and inactivation of LuxO. The P in the circle denotes that phosphorelay is the mechanism of signal transduction throughout the entire circuit. A periplasmic-binding protein called LuxP is hypothesised to interact with LuxQ to recognise AI-2 (not shown).

attachment to host cells, defensive factors to avoid elimination by the host, and factors that promote bacterial internalisation and host cell apoptosis [28,47]. Furthermore, the autoinducers produced by different *S. aureus* strains vary. These autoinducers specifically induce Agr-mediated quorum sensing in the strains that produce them and inhibit Agr-mediated quorum sensing in *S. aureus* strains that produce a different autoinducer [48,49••].

Quorum sensing via HSL autoinducer signalling has been shown to play a critical role in the proper development of bacterial biofilms [50•,51]. In biofilms, bacteria are organised into elaborate structures that can be composed of single or multiple species. Biofilms possess aqueous channels that promote the flow of nutrients and prevent desiccation. Bacteria localised to different regions of the structure display specialised patterns of gene expression and differentiation. Furthermore, biofilms are highly resistant to antibiotics [52,53]. These features of biofilms indicate that the bacteria in them have increased their chances of survival and proliferation by virtue of communal living (for further details on biofilms see the review by Pratt and Kolter, this issue, pp 598–603).

In another example of inter-species communication, quorum sensing regulates the production of the antibiotic phenazine in the plant pathogen *Pseudomonas aureofacien*s. Antibiotic production is controlled not only by the *P. aureofaciens* HSL autoinducer, but also by signals secreted by a number of other plant-associated bacterial species [54•]. These observations suggest that *P. aureofaciens* can detect situations in which intense competition for nutrients exists. Apparently, *P. aureofaciens* responds to this circumstance by producing phenazine to eliminate competitor bacteria.

In bacterial–eukaryotic interactions in which quorum sensing regulates processes deleterious to the host, one mechanism of host defence could be the production of antagonists that interfere with autoinducer reception. One striking example of this type of host response occurs in the seaweed *Delisea pulchra*. This organism produces a number of halogenated furanones and enones that interfere with HSL-mediated processes such as swarming in *Serratia liquefaciens* [55–57]. The structures of these anti-colonisation factors strongly resemble HSLs. In a recent study, the *D. pulchra* furanones have been shown to directly bind to the HSL-binding site in LuxR and to displace the cognate HSL autoinducer. Inhibition of quorum sensing was proportional to the ability of a given furanone to compete with the HSL autoinducer for binding [58••].

Conclusions

Considerable progress has been made this past year in our understanding of the variety of functions controlled by quorum sensing and the different mechanisms that bacteria use for counting cell number and modulating gene expression in response to changes in cell-population density. It is now clear that quorum sensing regulates bacterial communication in test tubes and in nature. It is also clear that intra- and inter-species cell–cell communication occurs and is regulated by quorum sensing systems. Further, there is mounting data demonstrating that autoinducer signals elicit specific responses from eukaryotic hosts. Emphasis should be placed on developing rigorous analyses of how bacteria communicate

within and between species, and on how eukaryotic hosts talk back. Several model quorum-sensing systems currently offer the possibility for such studies. Therefore, it is no longer sufficient to identify the next LuxI/LuxR system. The key now for understanding these complex and fascinating bacterial languages is to decipher the impact of the words.

Acknowledgements

This work was supported by the National Science Foundation Grant Number MCB-9506033 and The Office of Naval Research Grant number N00014-99-0767.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Nealson KH, Platt T, Hastings JW: **Cellular control of the synthesis and activity of the bacterial luminescent system.** *J Bacteriol* 1970, **104**:313-322.

2. Nealson KH, Hastings JW: **Bacterial bioluminescence: its control and ecological significance.** *Microbiol Rev* 1979, **43**:496-518.

3. Eberhard A, Burlingame AL, Eberhard C, Kenyon GL, Nealson KH, Oppenheimer NJ: **Structural identification of autoinducer of *Photobacterium fischeri* luciferase.** *Biochemistry* 1981, **20**:2444-2449.

4. Cao J, Meighen EA: **Purification and structural identification of an autoinducer for the luminescence system of *Vibrio harveyi*.** *J Biol Chem* 1989, **264**:21670-21676.

5. Engebrecht J, Nealson K, Silverman M: **Bacterial bioluminescence: isolation and genetic analysis of functions from *Vibrio fischeri*.** *Cell* 1983, **32**:773-781.

6. Engebrecht J, Silverman M: **Identification of genes and gene products necessary for bacterial bioluminescence.** *Proc Natl Acad Sci USA* 1984, **81**:4154-4158.

7. Engebrecht J, Silverman M: **Nucleotide sequence of the regulatory locus controlling expression of bacterial genes for bioluminescence.** *Nucleic Acids Res* 1987, **15**:10455-10467.

8. Fuqua WC, Winans SC, Greenberg EP: **Quorum sensing in bacteria: the LuxR–LuxI family of cell density-responsive transcriptional regulators.** *J Bacteriol* 1994, **176**:269-275.

9. Swift S, Williams P, Stewart GSAB: **N-acylhomoserine lactones and quorum sensing in proteobacteria.** In *Cell–Cell Signaling in Bacteria*. Edited by Dunny GM, Winans SC. Washington DC: ASM Press; 1999:291-313.

10. Passador L, Cook JM, Gambello MJ, Rust L, Iglewski BH: **Expression of *Pseudomonas aeruginosa* virulence genes requires cell to cell communication.** *Science* 1993, **260**:1127-1130.

11. Ochsner UA, Reiser J: **Autoinducer-mediated regulation of rhamnolipid biosurfactant synthesis in *Pseudomonas aeruginosa*.** *Proc Natl Acad Sci USA* 1995, **92**:6424-6428.

12. De Kievit T, Seed PC, Nezezon J, Passador L, Iglewski BH: **RsaL, a novel repressor of virulence gene expression in *Pseudomonas aeruginosa*.** *J Bacteriol* 1999, **181**:2175-2184.

13. You Z, Fukushima J, Tanaka K, Kawamoto S, Okuda K: **Induction of entry into the stationary growth phase in *Pseudomonas aeruginosa* by *N*-acylhomoserine lactone.** *FEMS Microbiol Lett* 1998, **164**:99-106.

14. Parsek MR, Val DL, Hanzelka BL, Cronan JE Jr, Greenberg EP: **Acyl homoserine-lactone quorum-sensing signal generation.** *Proc Natl Acad Sci USA* 1999, **96**:4360-4365.

15. Glessner A, Smith RS, Iglewski BH, Robinson JB: **Roles of *Pseudomonas aeruginosa las* and *rhl* quorum-sensing systems in control of twitching motility.** *J Bacteriol* 1999, **181**:1623-1629.

16. Schell MA: **To be or not to be: how *Pseudomonas solanacearum* decides whether or not to express virulence genes.** *Eur J Plant Pathol* 1996, **102**:459-469.

17. Flavier AB, Ganova-Raeva LM, Schell MA, Denny TP: **Hierarchical autoinduction in *Ralstonia solanacearum*: control of acyl-homoserine lactone production by a novel autoregulatory system responsive to 3-hydroxy-palmitic acid methyl ester.** *J Bacteriol* 1997, **179**:7089-7097.

18. • Flavier AB, Schell MA, Denny TP: **An RpoS (σ^s) homologue regulates acylhomoserine lactone-dependent autoinduction in *Ralstonia solanacearum*.** *Mol Microbiol* 1998, **28**:475-486.
Transposon mutagenesis was used to identify genes required for homoserine latone (HSL) production in *R. solanacearum*. The *rpoS* homologue was identified, and shown to be required for expression of the *luxR* homologue *solR*. SolR, in turn, is necessary for expression of the *luxI* homologue *solI*. RpoS exerts its effect on SolR–SolI independently of the LysR homologue PhcA. These results demonstrate that multiple regulatory pathways converge to control quorum sensing in *R. solanacearum*.

19. Oger P, Kim K-S, Sackett RL, Piper KR, Farrand SK: **Octopine-type Ti plasmids code for a mannopine-inducible dominant-negative allele of *traR*, the quorum-sensing activator that regulates Ti plasmid conjugal transfer.** *Mol Microbiol* 1998, **27**:277-288.

20. • Zhu J, Winans SC: **Activity of the quorum-sensing regulator TraR of *Agrobacterium tumefaciens* is inhibited by a truncated, dominant defective TraR-like protein.** *Mol Microbiol* 1998, **27**:289-297.
This report shows that a TraR-like protein called TraS is encoded on the *A. tumefaciens* virulence plasmid. TraS lacks a DNA-binding domain and acts as a dominant negative inhibitor of TraR function presumably by forming inactive mixed TraR–TraS oligomers. Expression of *traS* is induced by the plant hormone mannopine, which results in inhibition of TraR-mediated conjugation.

21. Winans SC, Zhu J, More MI: **Cell density-dependent gene expression by *Agrobacterium tumefaciens* during colonization of crown gall tumors.** In *Cell–Cell Signaling in Bacteria*. Edited by Dunny GM, Winans SC. Washington DC: ASM Press; 1999:117-128.

22. Reverchon S, Bouillant ML, Salmond G, Nasser W: **Integration of the quorum-sensing system in the regulatory networks controlling virulence factor synthesis in *Erwinia chrysanthemi*.** *Mol Microbiol* 1998, **29**:1407-1418.

23. Evans K, Passador L, Srikumar R, Tsang E, Nezezon J, Poole K: **Influence of the MexAB-OprM multidrug efflux system on quorum sensing in *Pseudomonas aeruginosa*.** *J Bacteriol* 1998, **180**:5443-5447.

24. Cha C, Gao P, Chen Y-C, Shaw PD, Farrand SK: **Production of acyl-homoserine lactone quorum-sensing signals by Gram-negative plant-associated bacteria.** *Mol Plant–Microbe Interactions* 1998, **11**:1119-1129.

25. Plamann L, Kaplan HB: **Cell-density sensing during early development in *Myxococcus xanthus*.** In *Cell–Cell Signaling in Bacteria*. Edited by Dunny GM, Winans SC. Washington DC: ASM Press; 1999:67-82.

26. Shimkets LJ, Kaiser D: **Cell contact-dependent C signaling in *Myxococcus xanthus*.** In *Cell–Cell Signaling in Bacteria*. Edited by Dunny GM, Winans SC. Washington DC: ASM Press; 1999:83-100.

27. Kleerebezem M, Quadri LEN, Kuipers OP, de Vos WM: **Quorum sensing by peptide pheromones and two-component signal-transduction systems in Gram-positive bacteria.** *Mol Microbiol* 1997, **24**:895-904.

28. Novick RP, Muir TW: **Virulence gene regulation by peptides in staphylococci and other Gram-positive bacteria.** *Curr Opin Microbiol* 1999, **2**:40-45.

29. Lazazzera BA, Grossman AD: **The ins and outs of peptide signaling.** *Trends Microbiol* 1998, **6**:288-294.

30. • Turgay K, Hahn J, Burghoorn J, Dubnau D: **Competence in *Bacillus subtilis* is controlled by regulated proteolysis of a transcription factor.** *EMBO J* 1998, **17**:6730-6738.
This study presents both *in vivo* and *in vitro* evidence that accumulation of the *B. subtilis* competence regulator ComK is controlled by MecA/ClpP driven proteolysis. The experiments show that the ComS protein, which is synthesised in response to the ComX and CSF quorum sensing peptides, allows ComK to dissociate from a complex with MecA and ClpP and escape degradation. The results demonstrate that this is a critical step in competence development.

31. Clewell DB: **Sex pheromone systems in *Enterococci*.** In *Cell–Cell Signaling in Bacteria*. Edited by Dunny GM, Winans SC. Washington DC: ASM Press; 1999:47-66.

32. Fujimoto S, Clewell DB: **Regulation of the pAD1 sex pheromone response of *Enterococcus faecalis* by direct interaction between the cAD1 peptide mating signal and the negatively regulating**

DNA-binding TraA protein. *Proc Natl Acad Sci USA* 1998, **95**:6430-6435.

33. do Carmo de Freire Bastos M, Tomita H, Tanimoto K, Clewell DB: **Regulation of the *Enterococcus faecalis* pAD1-related sex pheromone response: analyses of *traD* expression and its role in controlling conjugation functions.** *Mol Microbiol* 1998, **30**:381-392.

34. Van Wamel WJB, van Rossum G, Verhoef J, Vandenbroucke-Grauls CMJE, Fluit AC: **Cloning and characterization of an accessory gene regulator (*agr*)-like locus from *Staphylococcus epidermidis*.** *FEMS Mircobiol Lett* 1998, **163**:1-9.

35. Otto, M, Sussmuth R, Jung G, Gotz F: **Structure of the pheromone peptide of the *Staphylococcus epidermidis agr* system.** *FEBS Lett* 1998, **424**:89-94.

36. Lina G, Jarraud S, Ji G, Greenland T, Pedraza A, Etienne J, Novick RP, Vandenesch F: **Transmembrane topology and histidine protein kinase activity of AgrC, the *agr* signal receptor in *Staphylococcus aureus*.** *Mol Microbiol* 1998, **28**:655-662.

37. Bassler BL, Wright M, Showalter ME, Silverman MR: **Intercellular signalling in *Vibrio harveyi*: sequence and function of genes regulating expression of luminescence.** *Mol Microbiol* 1993, **9**:773–786.

38. Bassler BL, Wright M, Silverman MR: **Multiple signalling systems controlling expression of luminescence in *Vibrio harveyi*: sequence and function of genes encoding a second sensory pathway.** *Mol Microbiol* 1994, **13**:273-286.

39. Bassler BL, Wright M, Silverman MR: **Sequence and function of *luxO*, a negative regulator of luminescence in *Vibrio harveyi*.** *Mol Microbiol* 1994, **12**:403-412.

40. Bassler BL: **A multichannel two-component signaling relay controls quorum sensing in *Vibrio harveyi*.** In *Cell–Cell Signaling in Bacteria*. Edited by Dunny GM, Winans SC. Washington DC: ASM Press; 1999:259-273.

41. •• Freeman JA, Bassler BL: **A genetic analysis of the function of LuxO, a two-component response regulator involved in quorum sensing in *Vibrio harveyi*.** *Mol Microbiol* 1999, **31**:665-677.

This paper presents a genetic analysis of the mechanism of quorum sensing signal processing in *V. harveyi*. The experiments show that the two autoinducer sensors, LuxN and LuxQ, act both as kinases and phosphatases to control the concentration of LuxO–phosphate in the cell. Multiple locked missense alleles of LuxO were constructed and analysed to determine that LuxO–phosphate is the active Lux regulator and unphosphorylated LuxO has no activity. Taken together the results of these analyses demonstrate how *V. harveyi* precisely modulates light production over a 10^5-fold range.

42. • Freeman JA, Bassler BL: **Sequence and function of LuxU: a two component phosphorelay protein that regulates quorum sensing in *Vibrio harveyi*.** *J Bacteriol* 1999, **181**:899-906.

In this work, a new member of the *V. harveyi* Lux signal relay, LuxU, is identified and analysed. This paper presents the cloning, sequencing and mutagenesis of the LuxU phosphorelay protein. Importantly, the results demonstrate that the function of LuxU is to couple signals emanating from the two autoinducer sensors (LuxN and LuxQ) to the response regulator LuxO.

43. •• Surette MG, Miller MB, Bassler BL: **Quorum sensing in *Escherichia coli*, *Salmonella typhimurium* and *Vibrio harveyi*: a new family of genes involved in autoinducer production.** *Proc Natl Acad Sci USA* 1999, **96**:1639-1644.

This important work presents the identification and analysis of the *luxS* gene and demonstrates that it is responsible for AI-2 production in *V. harveyi*, *E. coli* and *S. typhimurium*. The paper further shows that very highly conserved *luxS* homologues are widespread in both Gram-negative and Gram-positive bacteria. These results imply that AI-2 signalling could be a universal cell–cell communication system.

44. •• Surette MG, Bassler BL: **Quorum sensing in *Escherichia coli* and *Salmonella typhimurium*.** *Proc Natl Acad Sci USA* 1998, **95**:7046-7050.

This paper presents the first convincing evidence for quorum sensing in *E. coli* and *S. typhimurium*. *V. harveyi* reporter strains that specifically respond to either AI-1 or AI-2 are used to show that *E. coli* and *S. typhimurium* produce an AI-2-like activity. Furthermore, this report shows that signal production in *E. coli* and *S. typhimurium* is highly regulated, responsive to a number of environmental cues, and autoinducer activity is degraded as the cells enter stationary phase. This is the first report of degradation of an autoinducer.

45. Surette MG, Bassler BL: **Regulation of autoinducer production in *Salmonella typhimurium*.** *Mol Microbiol* 1999, **31**:585-595.

46. Bassler BL, Greenberg EP, Stevens AM: **Cross-species induction of luminescence in the quorum sensing bacterium *Vibrio harveyi*.** *J Bacteriol* 1997, **179**:4043-4045.

47. Wesson CA, Liou LE, Todd KM, Bohach GA, Trumble WR, Bayles KW: ***Staphylococcus aureus* Agr and Sar global regulators influence internalization and induction of apoptosis.** *Infect Immun* 1998, **66**:5238-5243.

48. Ji G, Beavis R, Novick RP: **Bacterial interference caused by autoinducing peptide variants.** *Science* 1997, **276**:2027-2030.

49. •• Mayville P, Ji G, Beavis R, Yang H, Goger M, Novick RP, Muir TW: **Structure-activity analysis of synthetic autoinducing thiolactone peptides from *Staphylococcus aureus* responsible for virulence.** *Proc Natl Acad Sci USA* 1999, **96**:1218-1223.

The structure of the *S. aureus* autoinducer is reported. The autoinducers are shown to be cyclic thiolactone peptides. In a continuation of the studies begun in [48], the present work offers evidence that the thiolactone is required for induction but not inhibition of *agr* expression.

50. • Davies DG, Parsek MR, Pearson JP, Iglewski BH, Costerton JW, Greenberg EP: **The involvement of cell-to-cell signals in the development of a bacterial biofilm.** *Science* 1998, **280**:295-299.

This manuscript presents evidence that homoserine lactone (HSL) quorum sensing is involved in biofilm formation in *P. aeruginosa*. A *P. aeruginosa lasI* mutant that is incapable of production of the HSL autoinducer ($3OC_{12}$-HSL) is shown to be defective in biofilm formation. The mutant forms thinner biofilms than the wild type, and it does not form the microcolonies that are characteristic of wild-type biofilms. Exogenous addition of $3OC_{12}$-HSL complemented the defect.

51. Allison DG, Ruiz B, SanJose C, Jaspe A, Gilbert P: **Extracellular products as mediators of the formation and detachment of *Pseudomonas fluorescens* biofilms.** *FEMS Microbiol Lett* 1998, **167**:179-184.

52. Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM: **Microbial biofilms.** In *Annual Review of Microbiology*. Edited by Ornston LN, Balows A, Greenberg EP. Palo Alto CA: Annual Reviews; 1995:711-745.

53. O'Toole GA, Kolter R: **Initiation of biofilm formation in *Pseudomonas fluorescens* WCS365 proceeds via multiple, convergent signalling pathways: a genetic analysis.** *Mol Microbiol* 1998, **28**:449-461.

54. • Pierson EA, Wood DW, Cannon JA, Blachere FM, Pierson LS III: **Interpopulation signaling via N-acyl-homoserine lactones among bacteria in the wheat rhizosphere.** *Mol Plant–Microbe Interactions* 1998, **11**:1078-1084.

Naturally occurring wheat-rhizosphere associated bacteria are shown to restore phenazine production to a *P. aureofaciens* autoinducer mutant. This result implies that cross-feeding of quorum sensing autoinducers occurs in natural soil environments.

55. Lindum PW, Anthoni U, Christophersen C, Eberl L, Molin S, Givskov M: **N-acyl-L-homoserine lactone autoinducers control production of an extracellular lipopeptide biosurfactant required for swarming motility of *Serratia liquefaciens* MG1.** *J Bacteriol* 1998, **180**:6384-6388.

56. Givskov M, Ostling J, Eberl L, Lindum PW, Beck Christensen A, Christiansen G, Molin S, Kjelleberg S: **Two separate regulatory systems participate in control of swarming motility of *Serratia liquefaciens* MG1.** *J Bacteriol* 1998, **180**:742-745.

57. Givskov M, de Nys R, Manefield M, Gram L, Maximilien R, Eberl L, Molin S, Steinberg PD, Kjelleberg S: **Eukaryotic interference with homoserine lactone-mediated prokaryotic signalling.** *J Bacteriol* 1996, **178**:6618-6622.

58. •• Manefield M, de Nys R, Kumar N, Read R, Givskov M, Steinberg P, Kjelleberg S: **Evidence that halogenated furanones from *Delisea pulchra* inhibit acylated homoserine lactone (AHL)-mediated gene expression by displacing the AHL signal from its receptor protein.** *Microbiology* 1999, **145**:283-291.

In an extension of the work presented in [57], this paper presents evidence that the halogenated furanones produced by *D. pulchra* inhibit quorum sensing by competing for the homoserine-lactone (HSL) autoinducer-binding site in LuxR. Furanone-mediated inhibition of light production was monitored in an *E. coli* strain harbouring the *V. fischeri luxR* and *luxCDABE* genes. Furanones were further tested for the ability to displace radiolabelled *V. fischeri* autoinducer from *E. coli* cells overproducing LuxR. This important work, along with [57], suggests that one defensive strategy employed by eukaryotes to avoid colonisation by bacteria is to specifically target and inhibit quorum sensing controlled functions.