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QUORUM SENSING IN BACTERIA

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■ **Abstract** Quorum sensing is the regulation of gene expression in response to fluctuations in cell-population density. Quorum sensing bacteria produce and release chemical signal molecules called autoinducers that increase in concentration as a function of cell density. The detection of a minimal threshold stimulatory concentration of an autoinducer leads to an alteration in gene expression. Gram-positive and Gram-negative bacteria use quorum sensing communication circuits to regulate a diverse array of physiological activities. These processes include symbiosis, virulence, competence, conjugation, antibiotic production, motility, sporulation, and biofilm formation. In general, Gram-negative bacteria use acylated homoserine lactones as autoinducers, and Gram-positive bacteria use processed oligo-peptides to communicate. Recent advances in the field indicate that cell-cell communication via autoinducers occurs both within and between bacterial species. Furthermore, there is mounting data suggesting that bacterial autoinducers elicit specific responses from host organisms. Although the nature of the chemical signals, the signal relay mechanisms, and the target genes controlled by bacterial quorum sensing systems differ, in every case the ability to communicate with one another allows bacteria to coordinate the gene expression, and therefore the behavior, of the entire community. Presumably, this process bestows upon bacteria some of the qualities of higher organisms. The evolution of quorum sensing systems in bacteria could, therefore, have been one of the early steps in the development of multicellularity.

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INTRODUCTION

Quorum sensing was discovered and described over 25 years ago in two luminous marine bacterial species, *Vibrio fischeri* and *Vibrio harveyi* (104). In both species the enzymes responsible for light production are encoded by the luciferase structural operon *luxCDABE* (32, 98), and light emission was determined to occur only at high cell-population density in response to the accumulation of secreted autoinducer signaling molecules (104). Until recently, only a few other cases of bacterial regulation of gene expression in response to cell-cell signaling were known. For example, antibiotic production by *Streptomyces* spp. (21), conjugation in *Enterococcus faecalis* (25), and fruiting body development in *Myxococcus xanthus* (27) were also recognized to be controlled by intercellular signaling. These bacterial communication systems were considered anomalous, and in general, bacteria as a whole were not believed to use cell-cell communication. Rather, the exchange of chemical signals between cells/organisms was assumed to be a trait highly characteristic of eukaryotes. The recent explosion of advances in the field of cell-cell communication in bacteria has now shown that many or most bacteria probably communicate using secreted chemical molecules to coordinate the behavior of the group. Furthermore, we now know that a vast assortment of different classes of chemical signals are employed, that individual species of bacteria use more than one chemical signal and/or more than one type of signal to communicate, that complex hierarchical regulatory circuits have evolved to integrate and process the sensory information, and that the signals can be used to differentiate between species in consortia. It seems clear now that the ability to communicate both within and between species is critical for bacterial survival and interaction in natural habitats.

GRAM-NEGATIVE BACTERIA: LUXI/LUXR-TYPE QUORUM SENSING

In the past decade quorum sensing circuits have been identified in over 25 species of Gram-negative bacteria (for review see 5, 22, 41, 111). In every case except those of *V. harveyi* and *M. xanthus* the quorum sensing circuits identified in Gram-negative bacteria resemble the canonical quorum sensing circuit of the symbiotic bacterium *V. fischeri*. Specifically, these Gram-negative bacterial quorum sensing circuits contain, at a minimum, homologues of two *V. fischeri* regulatory proteins called LuxI and LuxR. The LuxI-like proteins are responsible for the biosynthesis of a specific acylated homoserine lactone signaling molecule (HSL) known as an autoinducer. The autoinducer concentration increases with increasing cell-population density. The LuxR-like proteins bind cognate HSL autoinducers that have achieved a critical threshold concentration, and the LuxR-autoinducer complexes also activate target gene transcription (31–33). Using this quorum sensing mechanism, Gram-negative bacteria can efficiently couple gene expression to fluctuations in cell-population density. Among the 25 species of bacteria that mediate quorum sensing by means of a LuxI/LuxR-type circuit, the *V. fischeri*, *Pseudomonas aeruginosa*, *Agrobacterium tumefaciens*, and *Erwinia carotovora* systems are the best understood, and descriptions of each of these systems are provided below. In these descriptions we have attempted to point out both the similarities and the differences between the regulatory networks. Presumably, differences in regulation of the various LuxI/LuxR-like circuits reflect the evolution of the progenitor LuxI/LuxR circuit to modern networks that are precisely adapted for a particular bacterium living in a specialized niche.

The *Vibrio fischeri* LuxI/LuxR Bioluminescence System

The most intensely studied quorum sensing system is that of the bioluminescent marine bacterium *V. fischeri*. This bacterium lives in symbiotic association with a number of eukaryotic hosts. In each case the host has developed a specialized light organ that is inhabited by a pure culture of a specific strain of *V. fischeri* at very high cell density. In these symbiotic associations the eukaryotic host supplies *V. fischeri* with a nutrient-rich environment in which to live. The role of *V. fischeri* is to provide the host with light (for review see 135, 136, 163). Each eukaryotic host uses the light provided by the bacteria for a specific purpose. For example, in the squid *Euprymna scolopes–V. fischeri* association, the squid has evolved an antipredation strategy in which it counter-illuminates itself using the light from *V. fischeri*. Counter-illumination enables the squid to avoid casting a shadow beneath it on bright clear nights when the light from the moon and stars penetrates the seawater (136, 163). In contrast, the fish *Monocentris japonicus* uses the light produced by *V. fischeri* to attract a mate. In this case two luminescent regions exist on the fish that are apparently seductive to fish of the opposite sex. Other uses for the *V. fischeri* light, such as warding off predators and attracting prey, have also been documented (104).

Regardless of the purpose for which the eukaryotic host has adapted the light, the regulation of light production by *V. fischeri* in the specialized light organs is identical. Light emission is tightly correlated with the cell-population density of the bacterial culture in the organ, and this phenomenon is controlled by quorum sensing. In the light organ the *V. fischeri* culture grows to extremely high cell densities, reaching 10^{11} cells per ml (107). As the *V. fischeri* culture grows, it produces and releases an autoinducer hormone into the extracellular environment, and the hormone is trapped inside the light organ with the bacteria. The specialized eukaryotic light organ is the only niche in which the autoinducer molecule is predicted to accumulate to any significant concentration and thus act as a signal. Accumulation of the autoinducer is assumed to communicate to the bacteria that they are "inside" the host as opposed to "outside" in the seawater. Detection of the autoinducer by *V. fischeri* elicits a signaling cascade that culminates in the emission of light (31). Thus, the quorum sensing system of *V. fischeri* has evolved to specifically enable the bacteria to produce light only under conditions in which there is a positive selective advantage for the light.

As mentioned above, the luciferase enzymes required for light production in *V. fischeri* are encoded by *luxCDABE*, which exists as part of the *luxICDABE* operon (31, 85). Two regulatory proteins called LuxI and LuxR comprise the quorum sensing apparatus. LuxI is the autoinducer synthase enzyme, and it acts in the production of an HSL, *N*-(3-oxohexanoyl)-homoserine lactone (28, 32). LuxR functions both to bind the autoinducer and to activate transcription of the *luxICDABE* operon (31, 55, 137, 149–151). Figure 1 shows the quorum sensing system of *V. fischeri*. Specifically, at low cell densities, the *luxICDABE* operon is transcribed at a low basal level. Therefore, a low level of autoinducer is produced (via *luxI*), and because the genes encoding luciferase are located directly downstream of the *luxI* gene, only a low level of light is produced (31). The HSL autoinducer is freely diffusible across the cell membrane, so the concentration of autoinducer in the extracellular environment is the same as the intracellular concentration of the autoinducer (73). As the *V. fischeri* culture grows, autoinducer accumulates to a threshold level ($\sim$1–10 μg/ml) that is sufficient for detection and binding by the cytoplasmic LuxR protein (28). Interaction of LuxR with the autoinducer unmasks the LuxR DNA binding domain, allowing LuxR to bind the *luxICDABE* promoter and activate its transcription (55). This action results in an exponential increase in both autoinducer production and light emission. The LuxR-HSL complex also acts to negatively regulate expression of *luxR*. This negative feedback loop is a compensatory mechanism that decreases *luxICDABE* expression in response to the positive feedback circuit (31).

The *Pseudomonas aeruginosa* LasI/LasR-RhlI/RhlR Virulence System

In the opportunistic human pathogen *P. aeruginosa*, a hierarchical LuxI/LuxR circuit regulates quorum sensing. Two pairs of LuxI/LuxR homologues, LasI/LasR

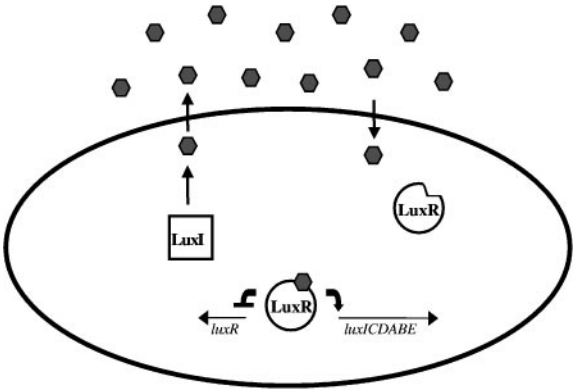

Figure 1 The *Vibrio fischeri* LuxI/LuxR quorum sensing circuit. There are five luciferase structural genes (*luxCDABE*) and two regulatory genes (*luxR* and *luxI*) required for quorum sensing–controlled light emission in *V. fischeri*. The genes are arranged in two adjacent but divergently transcribed units. *luxR* is transcribed to the left, and the *luxICDABE* operon is transcribed to the right. The LuxI protein (square) is responsible for synthesis of the HSL autoinducer *N*-(3-oxohexanoyl)-homoserine lactone (hexagons). As the cell-population density increases, the concentration of the autoinducer increases both intra- and extracellularly. At a critical autoinducer concentration, the LuxR protein (circle) binds the autoinducer. The LuxR-autoinducer complex binds at the *luxICDABE* promoter and activates transcription of this operon. This action results in an exponential increase in autoinducer synthesis via the increase in transcription of *luxI* and an exponential increase in light production via the increase in transcription of *luxCDABE*. The LuxR-autoinducer complex also binds at the *luxR* promoter, but in this case the complex represses the transcription of *luxR*. This negative action compensates for the positive action at the *luxICDABE* promoter. The oval represents a bacterial cell.

(113) and RhlI/RhlR (12), exist in *P. aeruginosa*. Both LasI and RhlI are autoinducer synthases that catalyze the formation of the HSL autoinducers *N*-(3-oxododecanoyl)-homoserine lactone (114) and *N*-(butryl)-homoserine lactone (115), respectively. The two regulatory circuits act in tandem to control the expression of a number of *P. aeruginosa* virulence factors.

The *P. aeruginosa* quorum sensing circuit functions as follows. At high cell density, LasR binds its cognate HSL autoinducer, and together they bind at promoter elements immediately preceding the genes encoding a number of secreted virulence factors that are responsible for host tissue destruction during initiation of the infection process. These pathogenicity determinants include elastase, encoded by *lasB*; a protease encoded by *lasA*; ExotoxinA, encoded by *toxA*; and alkaline phosphatase, which is encoded by the *aprA* gene (19, 22, 72, 113). Analogous to the *V. fischeri* LuxI/LuxR circuit, LasR bound to

autoinducer also activates *lasI* expression, which establishes a positive feedback loop (138).

The LasR-autoinducer complex also activates the expression of the second quorum sensing system of *P. aeruginosa* (108). Specifically, expression of *rhlR* is induced. RhlR binds the autoinducer produced by RhlI; this complex induces the expression of two genes that are also under the control of the LasI/LasR system, *lasB* and *aprA*. Additionally, the RhlR-autoinducer complex activates a second class of specific target genes. These genes include *rpoS*, which encodes the stationary phase sigma factor σ^s; *rhlAB*, which encodes rhamnosyltransferase and is involved in the synthesis of the biosurfactant/hemolysin rhamnolipid; genes involved in pyocyanin antibiotic synthesis; the *lecA* gene, which encodes a cytotoxic lectin; and the *rhlI* gene (12, 22, 41, 58, 82, 110, 111, 116, 121, 165, 166, 169). Again, similar to LasI/LasR and LuxI/LuxR, activation of *rhlI* establishes an autoregulatory loop.

As mentioned above, the LasR-autoinducer complex activates *rhlR* expression to initiate the second signaling cascade. However, the LasR-dependent autoinducer, *N*-(3-oxododecanoyl)-homoserine lactone, also prevents the binding of the RhlI-dependent autoinducer, *N*-(butryl)-homoserine lactone, to its cognate regulator RhlR (121). Presumably, this second level of control of RhlI/RhlR autoinduction by the LasI/LasR system ensures that the two systems initiate their cascades sequentially and in the appropriate order.

A novel, additional autoinducer has recently been demonstrated to be involved in quorum sensing in *P. aeruginosa*. This signal is noteworthy because it is not of the homoserine lactone class. Rather, it is 2-heptyl-3-hydroxy-4-quinolone (denoted PQS for *Pseudomonas* quinolone signal) (120). PQS partially controls the expression of the elastase gene *lasB* in conjunction with the Las and Rhl quorum sensing systems. The expression of PQS requires LasR, and PQS in turn induces transcription of *rhlI*. These data indicate that PQS is an additional link between the Las and Rhl circuits. The notion is that PQS initiates the Rhl cascade by allowing the production of the RhlI-directed autoinducer only after establishment of the LasI/LasR signaling cascade. A model showing the *P. aeruginosa* quorum sensing circuit is presented in Figure 2.

Recent studies on quorum sensing in *P. aeruginosa* have revealed that quorum sensing is crucial for proper biofilm formation. Specifically, *P. aeruginosa lasI* mutants do not develop into mature biofilms. Rather, they terminate biofilm formation at the micro-colony stage (19). These mutants can be complemented to wild-type biofilm production by the exogenous addition of the LasI-dependent HSL autoinducer *N*-(3-oxododecanoyl)-homoserine lactone. *P. aeruginosa* is the primary pathogen observed in the lungs of people afflicted with cystic fibrosis (CF), and microscopic analysis of CF sputum samples indicates that *P. aeruginosa* exists predominantly in biofilms in vivo. Finally, both the LasI- and the RhlI-directed autoinducers have been detected in sputum samples taken from CF patients (111, 143). These data indicate that biofilm formation by *P. aeruginosa* could be critical for colonization of the lung, and therefore antimicrobial therapies designed to

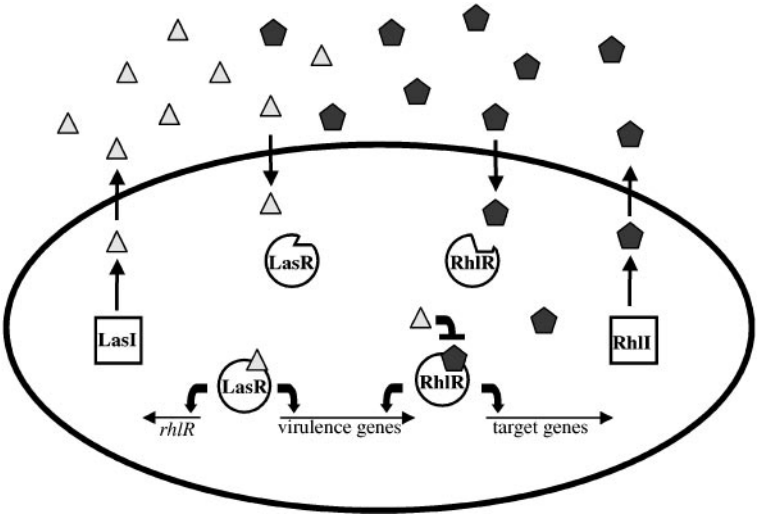

Figure 2 The *Pseudomonas aeruginosa* LasI/LasR-RhlI/RhlR quorum sensing system. *P. aeruginosa* uses two LuxI/LuxR-like autoinducer-sensor pairs for quorum sensing regulation of a variety of genes. The LasI protein produces the homoserine lactone signaling molecule *N*-(3-oxododecanoyl)-homoserine lactone (triangles), and the RhlI protein synthesizes *N*-(butryl)-homoserine lactone (pentagons). The LasR protein binds the LasI-dependent autoinducer when this signal molecule has accumulated to a critical threshold level. Subsequently, the LasR-autoinducer complex binds at a variety of virulence factor promoters and stimulates transcription (see text). Additionally, the LasR-autoinducer complex induces the transcription of *rhlR* to initiate the second quorum sensing circuit. RhlR bound to the RhlI-directed autoinducer activates the transcription of a subset of the LasR-activated virulence genes as well as several target genes that are not regulated by LasR. The LasI autoinducer interferes with binding of the RhlI-autoinducer to RhlR. Presumably, this action ensures that the LasI/LasR circuit is established prior to the establishment of the RhlI/RhlR circuit. The *Pseudomonas* quinolone signal (PQS) is an additional regulatory link between the Las and Rhl quorum sensing circuits (not shown, see text). The oval represents a bacterial cell. The squares represent the autoinducer synthase proteins LasI and RhlI, and the circles represent the transcriptional activator proteins LasR and RhlR.

interfere with quorum sensing, and by analogy with biofilm formation, could be used in the treatment of CF.

The *Agrobacterium tumefaciens* TraI/TraR Virulence System

A. tumefaciens is a plant pathogen that induces crown gall tumors on susceptible hosts. The transfer of the oncogenic Ti plasmid from the bacterium to the host

cell nucleus is required for the tumor formation process. Genes on the Ti plasmid direct the biosynthesis and secretion of opines in the plant. Subsequently, opines are consumed as a food source by the invading bacteria. The Ti plasmid also encodes genes that cause the production of phytohormones that induce host cell proliferation resulting in tumors (17, 141).

In *A. tumefaciens*, quorum sensing controls the conjugal transfer of the Ti plasmid between bacteria (124). Both the regulatory components TraI and TraR of the *A. tumefaciens* quorum sensing system are located on the transmissible Ti plasmid (42, 68, 174). Conjugation between *A. tumefaciens* cells requires two sensory signals, a host opine signal and the HSL autoinducer signal *N*-(3-oxoctanoyl)-homoserine lactone, which is the product of the bacterial TraI enzyme (23, 174). Opines produced at the site of the infection act both as a growth source for the bacteria and to initiate the quorum sensing cascade. Opines indirectly induce the expression of TraR via opine-specific regulators. There are two classes of opine-regulated conjugal Ti plasmids, octapine-type and nopaline-type. In octapine-type Ti plasmid transfer, the opine octapine acts to induce TraR via the activator OccR (40), whereas for nopaline-type Ti plasmids, the opines agrocinopine A and B induce TraR expression through the inactivation of the repressor AccR (11). Note that OccR and AccR are not LuxR homologues. Thus, quorum sensing in *A. tumefaciens* is responsive to both host and bacterial signals, indicating that this system has been well adapted for exclusive use at the host-pathogen interface.

At a primary level the *A. tumefaciens* quorum sensing circuit functions analogously to that of *V. fischeri*. Specifically, low, basal-level expression of *traI* results in low levels of autoinducer production. Following opine activation of the expression of *traR*, TraR binds the autoinducer, and the complex induces further expression of *traI* to establish the characteristic positive autoinduction loop. Target genes regulated by the autoinducer-TraR complex include the *tra* operon, the *trb* operon, and a gene called *traM* (38, 42, 68, 124). The *tra* operon is required for mobilization of the Ti plasmid, and the *trb* operon encodes the genes necessary for production of the mating pore. TraM, while induced by the TraR-autoinducer complex, acts to downregulate quorum sensing by binding to TraR and inhibiting TraR from binding DNA and activating target gene expression (38, 91). TraM therefore adds an additional level of regulation to the *A. tumefaciens* circuit that does not appear to exist in the *V. fischeri* circuit.

The *Erwinia carotovora* ExpI/ExpR-CarI/CarR Virulence/Antibiotic System

The pathogenic bacterium *E. carotovora* causes soft-rot in potato and other plant hosts. The secretion of cellulase and several pectinases that macerate the plant cell walls mediates pathogenicity in *E. carotovora* (61). A cognate pair of LuxI/LuxR homologues, ExpI/ExpR, is assumed to control secretion of the exoenzymes. Specifically, mutagenesis of *expI* results in a pleiotropic phenotype that affects several secreted enzymes (72, 125). No concrete role for ExpR in regulation of

the virulence factors has yet been established (2). Presumably, secretion of exoenzymes only at high cell density, under the control of a quorum sensing regulatory cascade, ensures that the bacteria do not prematurely wound the host and thus alert the host to their presence prior to achieving a sufficient bacterial cell number to mount a successful infection.

Similar to *P. aeruginosa*, a second LuxI/LuxR-like quorum sensing pair exists in *E. carotovora*. The CarI/CarR circuit regulates the biosynthesis of carbapenem antibiotics in response to cell-population density and an HSL autoinducer (4, 167). In *E. carotovora* antibiotic and exoenzyme production occur simultaneously. It is hypothesized that as the exoenzymes damage the plant cell wall, the antibiotic functions to fend off competitor bacteria that attempt to invade the plant by taking advantage of the wound produced by the *E. carotovora* enzymes. Both CarI and ExpI produce the identical HSL autoinducer, *N*-(3-oxohexanoyl)-homoserine lactone (2). This result indicates that CarR and ExpR respond to the same signal, which could effectively couple the timing of their individual regulatory activities.

Functions of the LuxI and LuxR Family of Proteins

In every described case, research indicates that the fundamental functions and biochemical mechanisms of action of the various LuxI-like and LuxR-like proteins are identical to those of LuxI and LuxR of *V. fischeri*. Research on *V. fischeri* LuxI and LuxR, as well as several of their homologues, has contributed to our understanding of HSL biosynthesis and detection and quorum sensing transcriptional activation. Below is a summary of information about the LuxI and LuxR protein families. Table 1 shows a list of bacterial species known to possess LuxI and/or LuxR proteins, the structure of the autoinducers, and their regulated functions if known.

LUXI FUNCTION *S*-adenosylmethionine (SAM) and acyl-acyl carrier protein (acyl-ACP) are the substrates for the LuxI-type enzymes. Acyl-ACP is an intermediate in fatty acid biosynthesis that is also acted on by the LuxI enzymes in HSL biosynthesis. The LuxI proteins couple a specific acyl-ACP to SAM via amide bond formation between the acyl side chain of the acyl-ACP and the amino group of the homocysteine moiety of SAM. The subsequent lactonization of the ligated intermediate in the reaction, along with the release of methylthioadenosine, results in the formation of the HSL autoinducer (56, 100, 161). Figure 3 shows a scheme for LuxI-directed HSL biosynthesis.

This biochemical mechanism for conversion of SAM and specific acyl-ACPs to HSL autoinducers has now been demonstrated for several LuxI-like proteins from different quorum sensing bacteria. These enzymes include LuxI from *V. fischeri* (56), TraI from *A. tumefaciens* (100), and RhlI from *P. aeruginosa* (112). Taken together, these results suggest that the SAM/acyl-ACP biosynthetic pathway is likely conserved among all LuxI homologues. Homoserine lactone autoinducers differ only in their respective acyl side chains. This fact suggests that

TABLE 1 Organisms possessing LuxI/LuxR homologues: the regulatory proteins, the HSL autoinducers, and the regulated functions[a]

Organism	LuxI/LuxR Homologue(s)	Autoinducer Identity	Target Genes and Functions
Vibrio fischeri	LuxI/LuxR	*N*-(3-oxohexanoyl)-HSL	*luxICDABE* (bioluminescence) (28, 31)
Aeromonas hydrophila	AhyI/AhyR	*N*-butanoyl-HSL	Serine protease and metalloprotease production (154)
Aeromonas salmonicida	AsaI/AsaR	*N*-butanoyl-HSL	*aspA* (exoprotease) (155)
Agrobacterium tumefaciens	TraI/TraR	*N*-(3-oxooctanoyl)-HSL	*tra*, *trb* (Ti plasmid conjugal transfer) (124, 174)
Burkholderia cepacia	CepI/CepR	*N*-octanoyl-HSL	Protease and siderophore production (87)
Chromobacterium violaceum	CviI/CviR	*N*-hexanoyl-HSL	Violacein pigment, hydrogen cyanide, antibiotics, exoproteases and chitinolytic enzymes (14, 96)
Enterobacter agglomerans	EagI/EagR	*N*-(3-oxohexanoyl)-HSL	Unknown (156)
Erwinia carotovora	(a) ExpI/ExpR (b) CarI/CarR	*N*-(3-oxohexanoyl)-HSL	(a) Exoenzyme synthesis, (72, 125) (b) Carbapenem antibiotic synthesis (4)
Erwinia chrysanthemi	ExpI/ExpR	*N*-(3-oxohexanoyl)-HSL	*pecS* (regulator of pectinase synthesis) (103, 132)
Erwinia stewartii	EsaI/EsaR	*N*-(3-oxohexanoyl)-HSL	Capsular polysaccharide biosynthesis, virulence (10)
Escherichia coli	?/SdiA	?	*ftsQAZ* (cell division), chromosome replication (44, 144, 170)
Pseudomonas aereofaciens	PhzI/PhzR	*N*-hexanoyl-HSL	phz (phenazine antibiotic biosynthesis) (123, 171)
Pseudomonas aeruginosa	(a) LasI/LasR	(a) *N*-(3-oxododecanoyl)-HSL	(a) *lasA*, *lasB*, *aprA*, *toxA* (exoprotease virulence factors), biofilm formation (19, 22 and references therein; 114)

TABLE 1 (*Continued*)

Organism	LuxI/LuxR Homologue(s)	Autoinducer Identity	Target Genes and Functions
	(b) RhlI/RhlR	(b) *N*-butyryl-HSL	(b) *lasB*, *rhlAB* (rhamnolipid), *rpoS* (stationary phase) (22 and references therein; 82, 115)
Ralstonia solanacearum	SolI/SolR	*N*-hexanoyl-HSL, *N*-octanoyl-HSL	Unknown (34)
Rhizobium etli	RaiI/RaiR	Multiple, unconfirmed	Restriction of nodule number (134)
Rhizobium leguminosarum	(a) RhiI/RhiR	(a) *N*-hexanoyl-HSL	(a) *rhiABC* (rhizosphere genes) and stationary phase (18, 51, 133)
	(b) CinI/CinR	(b) *N*-(3-hydroxy-7-*cis*-tetradecenoyl)-HSL	(b) Quorum sensing regulatory cascade (90)
Rhodobacter sphaeroides	CerI/CerR	7,8-*cis*-*N*-(tetradecanoyl)-HSL	Prevents bacterial aggregation (130)
Salmonella typhimurium	?/SdiA	?	*rck* (resistance to competence killing), ORF on *Salmonella* virulence plasmid (1)
Serratia liquefaciens	SwrI/?	*N*-butanoyl-HSL	Swarmer cell differentiation, exoprotease (30, 47)
Vibrio anguillarum	VanI/VanR	*N*-(3-oxodecanoyl)-HSL	Unknown (97)
Yersinia enterocolitica	YenI/YenR	*N*-hexanoyl-HSL, *N*-(3-oxohexanoyl)-HSL	Unknown (157)
Yersinia pseudotuberculosis	(a) YpsI/YpsR	(a) *N*-(3-oxohexanoyl)-HSL	Hierarchical quorum sensing cascade regulating bacterial aggregation and motility (3)
	(b) YtbI/YtbR	(b) *N*-octanoyl-HSL	

[a]Much of the information in this table comes from (22) with permission.

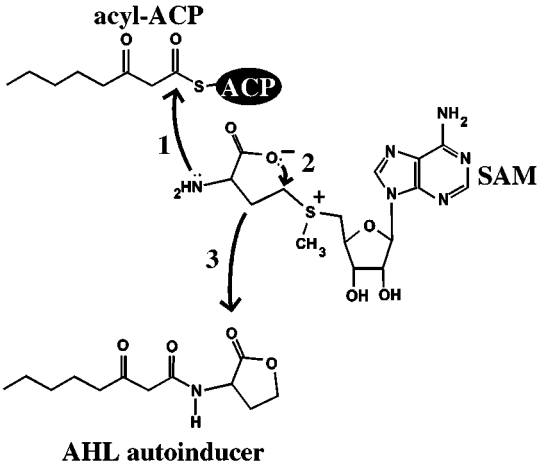

Figure 3 LuxI-directed biosynthesis of acylated homoserine lactone autoinducers. The LuxI family of proteins uses *S*-adenosylmethionine (SAM) and specific acyl-acyl carrier proteins (acyl-ACP) as substrates for HSL autoinducer biosynthesis. The LuxI-type proteins direct the formation of an amide linkage between SAM and the acyl moiety of the acyl-ACP (denoted 1). Subsequent lactonization of the ligated intermediate with the concomitant release of methylthioadenosine occurs (denoted 2). This step results in the formation of the acylated homoserine lactone (denoted 3). Shown in the figure is the HSL autoinducer *N*-(3-oxooctanoyl)-homoserine lactone, which is synthesized by TraI of *A. tumefaciens*. Figure courtesy of S.C. Winans.

the specificity for interaction of a particular LuxI-type protein with the correct acyl-ACP is encoded in the acyl side chain moiety of the acyl-ACP (39). A precise interaction between a certain LuxI-like protein and one specific acyl-ACP could provide a means for each LuxI-like protein to produce only one autoinducer. There are a few cases known in which a LuxI-like protein synthesizes more than one HSL. However, usually one HSL is the predominant species produced.

LUXR FUNCTION The LuxR-like proteins are each responsible for binding a cognate HSL autoinducer, binding specific target gene promoters, and activating transcription. Work performed with the *V. fischeri* LuxR protein shows that it consists of two domains. The amino-terminal domain is involved in binding to the HSL autoinducer, and the carboxyl-terminal domain is required for DNA binding and transcriptional activation (15, 16, 139, 145). The amino-terminal domain inhibits DNA binding by the carboxyl-terminal domain; this inhibitory function is eliminated when LuxR is bound to autoinducer. Residues in the carboxyl-terminal domain are also required for multimerization of the LuxR protein, and LuxR multimers are the species that binds promoter DNA sequences (149–151).

Results with TraR from *A. tumefaciens* strongly suggest that the TraR protein cannot fold in the absence of the autoinducer (176). A recent study shows that apo-TraR is sensitive to proteolytic degradation, whereas TraR, complexed with its cognate autoinducer, is resistant to proteolysis. The *A. tumefaciens* autoinducer confers resistance to proteolysis only to nascent TraR, but not to previously synthesized TraR protein. Interaction of TraR with its cognate autoinducer was shown to promote dimerization, and these TraR dimers bound promoter DNA (177). These results are exciting because they are the first example of a protein requiring its ligand for folding. Another very recent TraR study reports a different model for TraR autoinducer binding/gene activation. TraR is reported to be associated with the cell membrane. Binding of autoinducer causes TraR to form dimers that are released from the membrane and are therefore free to bind DNA to activate transcription (131).

As described, LuxR-like proteins also bind promoter DNA sequences. The carboxyl-terminal domain of each LuxR homologue contains a highly conserved helix-turn-helix motif that is responsible for DNA binding. In each case, the LuxR-type proteins bind a similar DNA promoter element termed the "lux box." The lux box consists of a 20-basepair palindromic DNA sequence situated at roughly -40 from the start site of transcription of a given target gene (43). Because the DNA recognition elements in the LuxR-type proteins are conserved, it is hypothesized that the target specificity inherent in these systems derives from the selectivity of a particular LuxR-type protein for its cognate autoinducer. Data supporting this assertion comes from the fact that LuxR homologues have been shown to be capable of activating the expression of noncognate target genes. Specifically, a particular LuxR-type protein bound to its cognate autoinducer can activate transcription by binding to a variety of lux boxes and activating the expression of the downstream heterologous gene (50, 164). Although the LuxIs produce a highly related family of molecules, in general, the HSL autoinducers are not capable of cross-stimulation of a noncognate system. Consequently, the LuxRs are extremely sensitive to alterations in the acyl side chains of the autoinducers. Evidence for this supposition comes from several studies demonstrating that only compounds that are closely related to the true autoinducer are capable of inducing weak/modest activation of gene expression, whereas compounds with less similarity are not active. Furthermore, in several cases autoinducer analogues inhibit cognate autoinducer binding to LuxR and, therefore, inhibit target gene activation (50, 137, 175).

GRAM-POSITIVE BACTERIA: PEPTIDE MEDIATED QUORUM SENSING

Gram-positive bacteria also regulate a variety of processes in response to increasing cell-population density. However, in contrast to Gram-negative bacteria, which use HSL autoinducers, Gram-positive bacteria employ secreted peptides as autoinducers for quorum sensing. In general, the peptide is secreted via a dedicated

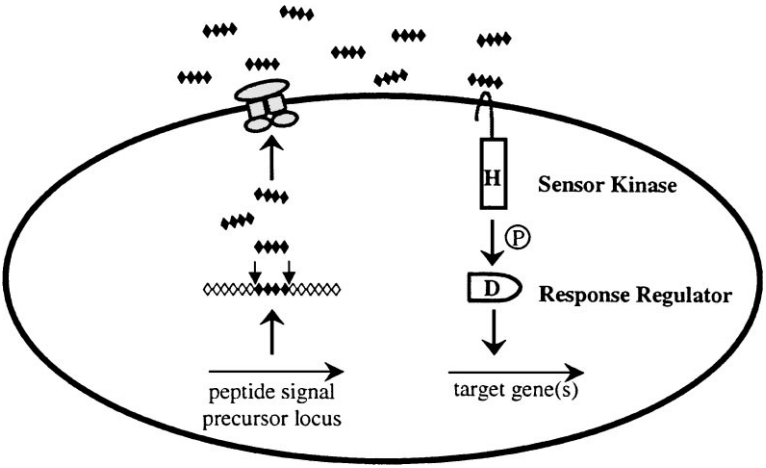

Figure 4 A general model for peptide-mediated quorum sensing in Gram-positive bacteria. In Gram-positive bacteria, a peptide signal precursor locus is translated into a precursor protein (black and white diamonds) that is cleaved (arrows) to produce the processed peptide autoinducer signal (black diamonds). Generally, the peptide signal is transported out of the cell via an ABC transporter (gray protein complex). When the extracellular concentration of the peptide signal accumulates to the minimal stimulatory level, a histidine sensor kinase protein of a two-component signaling system detects it. The sensor kinase autophosphorylates on a conserved histidine residue (H), and subsequently, the phosphoryl group is transferred to a cognate response regulator protein. The response regulator is phosphorylated on a conserved aspartate residue (D). The phosphorylated response regulator activates the transcription of target gene(s). The oval represents a bacterial cell. The "P" in the circle represents the phosphorylation cascade. Note that the lengths of the precursor and processed peptides are not meant to signify any specific number of amino acid residues.

ATP-binding cassette (ABC) transporter. Again, in contrast to the widespread use of LuxR-type proteins as autoinducer sensors by Gram-negative bacteria, Gram-positive bacteria use two-component adaptive response proteins for detection of the autoinducers. The signaling mechanism is a phosphorylation/dephosphorylation cascade (5, 76, 83; see 64 for a review of two-component signaling). A general model for quorum sensing in Gram-positive bacteria is shown in Figure 4. In brief, secreted peptide autoinducers increase in concentration as a function of the cell-population density. Two-component sensor kinases are the detectors for the secreted peptide signals. Interaction with the peptide ligand initiates a series of phosphoryl events that culminate in the phosphorylation of a cognate response regulator protein. Phosphorylation of the response regulator activates it, allowing it to bind DNA and alter the transcription of the quorum sensing–controlled target gene(s).

Several Gram-positive quorum sensing systems have been extensively studied. Here we describe the model systems controlling competence in *Streptococcus pneumoniae*, competence and sporulation in *Bacillus subtilis*, and virulence in *Staphylococcus aureus*. As described above for Gram-negative quorum sensing bacteria, in Gram-positive bacteria the fundamental signaling mechanism is conserved, but differences in regulation/timing of the systems have apparently arisen to heighten the effectiveness of the signal transduction process for a given environment.

The *Streptococcus pneumoniae* ComD/ComE Competence System

Genetic transformation in bacteria was originally described in *S. pneumoniae* (21). This process requires that the recipient bacterium become "competent" in order to acquire exogenous DNA molecules. The progression of events that results in *S. pneumoniae* (and *B. subtilis*) achieving the "competent state" is complex, and partial control of this phenomenon is via a well-studied quorum sensing system (60).

The peptide signal required for development of the competent state in *S. pneumoniae* is called CSP (competence stimulating peptide). CSP is a 17–amino acid peptide that is produced from a 41–amino acid precursor peptide called ComC (59, 129). The ComAB ABC transporter apparatus secretes processed CSP (66, 67). Detection of accumulated CSP at high cell density occurs via the ComD sensor kinase protein (122). High levels of CSP induce autophosphorylation of ComD and subsequent transfer of the phosphoryl group to the response regulator ComE. Phospho-ComE activates transcription of the *comX* gene. ComX is an alternative σ factor that is required for transcription of structural genes that are involved in the development of competence (86).

The *S. pneumoniae* competent state is transient and occurs only during exponential growth. In later stages of growth *S. pneumoniae* loses the ability to take up exogenous DNA (21, 65, 158). Therefore, other, as yet unidentified, regulators must exist that are responsible for transitioning *S. pneumoniae* out of the competent state. What is remarkable about competence in *S. pneumoniae* is that this bacterium is able to take up DNA irrespective of its sequence and therefore its species of origin. This quorum sensing–controlled process could allow *S. pneumoniae* to assimilate DNA only under conditions that would favor the likelihood of the presence of heterologous DNA that contain a collection of genes specifying novel functions that have not evolved in *S. pneumoniae*.

The *Bacillus subtilis* ComP/ComA Competence/ Sporulation System

B. subtilis is a soil organism that uses an elaborate peptide quorum sensing system to choose between development of the competent state and the sporulation process. Only 10% of a given population of *B. subtilis* cells become competent,

and in contrast to *S. pneumoniae*, the competent state is achieved at the transition between logarithmic and stationary phase growth (for review see 52, 83). Presumably, increased levels of exogenous DNA are available as the population enters stationary phase owing to cell lysis. Tuning the onset of the competent state to a later stage of growth (i.e. higher cell density) probably ensures that *B. subtilis* inherits its own species' DNA. It is hypothesized that the small subpopulation of cells that are competent for DNA uptake use the DNA they acquire as a repository of genetic material that can be exploited for repair of damaged/mutated chromosomes.

Sporulation in *B. subtilis* occurs when environmental conditions have deteriorated and the bacteria are depleted for nutrients. The bacteria undergo an asymmetric cell division, resulting in the formation of dormant, environmentally resistant spores (for review see 63). Sporulation occurs only poorly at low cell density, even if the *B. subtilis* cells are starved. Regulation of sporulation at high cell density requires several extracellular/environmental signals, but as described below, part of this control is certainly via a quorum sensing mechanism. Commitment to vegetative growth, competence, or the sporulation process is irreversible, and an incorrect choice by the *B. subtilis* cell would likely have fatal consequences.

Two peptides mediate quorum sensing control of competence and sporulation in *B. subtilis*. The peptides are called ComX and CSF (competence and sporulation factor). Both peptides are secreted and accumulate as the cell density increases. The ComX peptide is 10 amino acids long, and it contains a hydrophobic modification on a tryptophan residue that is required for signaling activity. The processed ComX peptide is derived from a 55–amino acid precursor peptide that is encoded by the *comX* gene (93, 146, 147). Although the machinery that is required for secretion of ComX has not been identified, a protein called ComQ is required for production of the peptide (93). The specific role of ComQ remains unknown. Detection of accumulated ComX signal is via the two-component ComP/ComA sensor kinase/response regulator pair (93, 147). Phospho-ComA activates the expression of the *comS* gene, and ComS inhibits the proteolytic degradation of the ComK protein (159). ComK is a transcriptional activator that controls the expression of structural genes required to develop competence (160, 162). ComK is subject to several modes of regulation, one of which is a transcriptional autoregulatory loop (54, 160). This positive feedback mechanism likely promotes the commitment of the cells to the competence pathway.

The second quorum sensing peptide in *B. subtilis*, CSF, is a pentapeptide. The five amino acids at the C-terminus of the precursor peptide PhrC are cleaved to form the CSF signal molecule (146). CSF accumulates extracellularly as a function of increasing cell density. However, the signaling role of CSF is intracellular. Extracellular CSF is imported into *B. subtilis* by an ABC-type oligopeptide transporter called Opp (84, 146, 147). When the intracellular concentration of CSF is low, CSF binds to and inhibits a ComA-specific phosphatase called RapC (118, 146). As mentioned, phospho-ComA is the response regulator controlling the expression of genes required for competence. Inhibition of RapC by CSF causes a

net increase in the level of phospho-ComA. Therefore, low levels of internal CSF promote competence development.

Whereas low internal concentrations of CSF promote competence, high internal levels of CSF inhibit competence and induce sporulation. At high concentration CSF inhibits the expression of *comS*, which results in increased proteolysis of ComK, the protein required for the decision to commit to competence (146). Furthermore, at high internal concentration, CSF promotes sporulation. The mechanism by which CSF stimulates sporulation is analogous to the mechanism for CSF stimulation of competence. In this case CSF inhibits a phosphatase called RapB. RapB dephosphorylates a response regulator (Spo0A) that is involved in promoting sporulation. Therefore, inhibition of the RapB phosphatase activity increases the levels of phospho-Spo0A, favoring a switch in commitment from competence to the sporulation pathway (52, 62, 118, 119). Presumably, adjustment of the concentration of CSF above or below some critical level tips the balance in favor of one lifestyle over another, allowing *B. subtilis* to correctly choose to commit to one of two very different terminal fates. Figure 5 shows the interconnected competence/sporulation quorum sensing pathway of *B. subtilis*.

The *Staphylococcus aureus* AgrC/AgrA Virulence System

The invasive pathogen *S. aureus* causes a variety of human illnesses, including skin infections, toxic shock syndrome, endocarditis, and food poisoning. This multitalented bacterium can promote disease in almost any tissue in the human body. Primarily compromised individuals are susceptible to *S. aureus* infection. A variety of pathogenicity determinants are required for successful invasion by *S. aureus*, many of which are regulated by peptide quorum sensing. (For a review of *S. aureus* pathogenesis see 105.)

An RNA molecule called RNAIII regulates density-dependent pathogenicity in *S. aureus* (69, 102). The *agrBDCA* operon encodes the components of a peptide quorum sensing system that is partially responsible for regulating the levels of RNAIII (101, 106, 117). The *agrBDCA* locus is adjacent to and transcribed divergently from the *hld* gene. The *hld* gene encodes a hemolysin, and *hld* also encodes the RNAIII transcript (69, 102). The *agrD* gene specifies the 46–amino acid precursor peptide AgrD that is processed to a final octapeptide by an AgrB-dependent mechanism (70, 71). The processed autoinducing peptide (AIP) contains a thiolactone ring that is absolutely required for full signaling activity (95). AgrC is the sensor kinase and AgrA is the cognate response regulator of the two-component system (89, 117). Phospho-AgrA, via an unknown mechanism, increases the concentration of RNAIII. RNAIII functions as an effector to activate the expression of an array of secreted virulence factors.

Tremendous strain-to-strain variation exists in the AIPs of *S. aureus*. The variability in the AIPs determines their specificity for interaction with a particular AgrC sensor kinase. *S. aureus* strains can be categorized into four different groups based on the specificity of their AIPs. A remarkable feature of the *S. aureus*

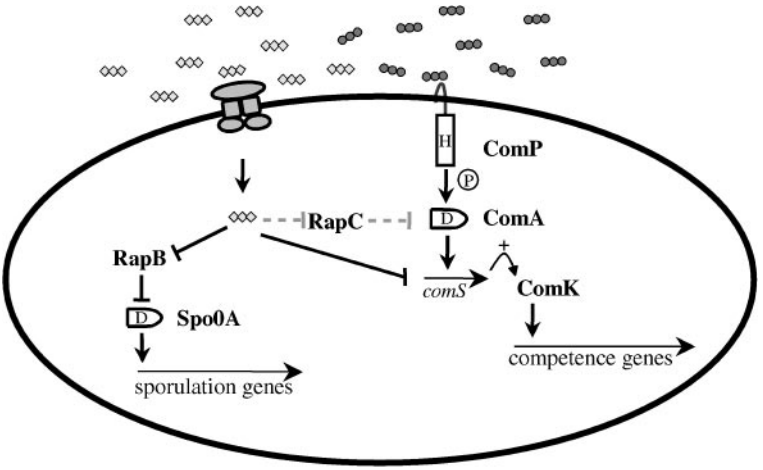

Figure 5 Quorum sensing control of competence and sporulation in *Bacillus subtilis*. *B. subtilis* employs two processed peptide autoinducers, ComX (gray circles) and CSF (white diamonds), to regulate the competence and sporulation processes. Refer to Figure 4 for the general features of the processing and export of these peptides. Accumulation of the processed ComX peptide enables it to interact with the ComP sensor kinase. ComP autophosphorylates on a histidine residue (H), and subsequently phosphate is transferred to an aspartate residue (D) on the ComA response regulator. Phospho-ComA activates the transcription of *comS*. The ComS protein increases the level of ComK protein (+) by inhibiting ComK proteolysis. ComK is a transcription factor that activates the expression of genes required for development of the competent state. The second peptide autoinducer, competence and sporulation factor (CSF), while accumulating extracellularly in a density-dependent manner, has an intracellular role. CSF is transported into the cell via the Opp transporter (gray protein complex). At low internal concentrations CSF inhibits the ComA-specific phosphatase RapC. Inhibition of RapC increases the level of phospho-ComA, which leads to competence (dashed lines). At high internal CSF concentrations, CSF inhibits competence and promotes spore development (black lines). Specifically, CSF inhibits ComS. CSF inhibition of ComS activity reduces transcription of competence genes, promoting sporulation instead. Additionally, CSF inhibits the RapB phosphatase. The role of RapB is to dephosphorylate the response regulator Spo0A. Phospho-Spo0A induces sporulation. Therefore, CSF inhibition of the RapB phosphatase increases the phospho-Spo0A levels, and this leads to sporulation.

quorum sensing system is that each AIP, while initiating the *agr* virulence cascade in its own group, specifically inhibits the *agr* response in the other *S. aureus* groups (70, 95, 109). It is hypothesized that this interference with virulence allows the invading *S. aureus* strain that is first to establish its quorum sensing circuit to out-compete secondary invading strains.

QUORUM SENSING IN *VIBRIO HARVEYI*: A HYBRID HOMOSERINE LACTONE/TWO-COMPONENT SIGNALING CIRCUIT

The *V. harveyi* quorum sensing circuit possesses features reminiscent of both Gram-negative and Gram-positive quorum sensing systems. Similar to other Gram-negative bacteria, *V. harveyi* produces and responds to an acylated homoserine lactone. In contrast to Gram-negative bacteria, but analogous to Gram-positive bacteria, quorum sensing signal transduction in *V. harveyi* occurs via a two-component circuit (5). Additionally, *V. harveyi* possesses a novel signaling molecule, denoted AI-2 (7, 8). This molecule and the gene required for its production have recently been shown to be present in a variety of Gram-negative and Gram-positive bacteria (152, 153). AI-2 could be the link that ties together the evolution of the two major classes of quorum sensing circuits.

The *V. harveyi* Multi-Channel Lux Circuit

Analogous to *V. fischeri*, *V. harveyi*, a related free-living marine bacterium, uses quorum sensing to regulate bioluminescence. In *V. harveyi* two quorum sensing systems function in parallel to control the density-dependent expression of the luciferase structural operon *luxCDABE*. Each system is composed of a sensor (Sensor 1 or Sensor 2) and its cognate autoinducer (AI-1 or AI-2) (7, 8). AI-1 is *N*-(3-hydroxybutanoyl)-homoserine lactone (13). However, unlike in *V. fischeri*, synthesis of this HSL autoinducer is not dependent on a LuxI-like protein. The LuxLM protein is required for production of AI-1 and it shares no homology to the LuxI family, although the biosynthetic pathway is probably identical (7). The structure of the second *V. harveyi* autoinducer (AI-2) is not known. AI-1 and AI-2 are detected by their cognate sensors LuxN (Sensor 1) and LuxQ (Sensor 2). LuxN and LuxQ are two-component proteins of the hybrid-sensor class. LuxN and LuxQ each contain a sensor kinase domain and a response regulator domain (7, 8). Another protein, LuxP, is required in conjunction with LuxQ to transduce the AI-2 signal. LuxP is similar to the ribose binding protein of *Escherichia coli* and *Salmonella typhimurium* (8). LuxN and LuxQ channel phosphate to a shared signal integrator protein called LuxU (36). LuxU is a phosphotransferase protein, which transfers the signal to a response regulator protein called LuxO (9, 35, 36).

Genetic analysis in *V. harveyi* suggests that at low cell density, in the absence of the autoinducers, the sensors autophosphorylate, and following a series of intra- and intermolecular phospho-transfer events, LuxO is phosphorylated. Phospho-LuxO causes repression of *luxCDABE* expression, but this effect is indirect (35–37). Recent evidence suggests that LuxO is a σ^{54}-dependent activator of an unidentified repressor of *luxCDABE* expression (88). At high cell density, in the presence of their autoinducing ligands, the sensors LuxN and LuxQ transition from kinases that drive phosphate toward LuxO to phosphatases that drain phosphate out of the circuit. Unphosphorylated LuxO does not activate the expression of the repressor,

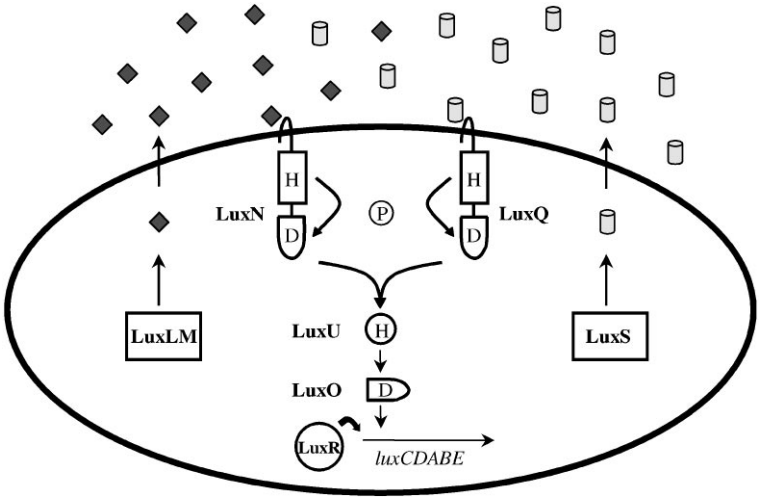

Figure 6 The hybrid HSL-two-component quorum sensing circuit of *Vibrio harveyi*. *V. harveyi* uses two autoinducers to regulate density-dependent light production. AI-1 is *N*-(3-hydroxybutanoyl)-homoserine lactone (diamonds), and AI-2 (cylinders) is of unknown structure. The proteins required for synthesis of AI-1 and AI-2 are LuxLM and LuxS, respectively. A cognate two-component sensor kinase protein detects each autoinducer. Specifically, LuxN is responsible for detection of AI-1 and LuxQ is responsible for AI-2. Additionally, a periplasmic binding protein called LuxP is required for AI-2 detection (not pictured). Both LuxN and LuxQ are hybrid sensor kinases that contain a sensor kinase domain and an attached response regulator domain. Signaling from both sensors is channeled to a shared integrator protein called LuxU. LuxU is a phosphotransferase protein that relays the sensory information to the response regulator LuxO. LuxO acts negatively to control the luciferase structural operon *luxCDABE*. A transcriptional activator called LuxR, which is not similar to the *V. fischeri* LuxR family, is also required for expression of *luxCDABE*. The circuit is proposed to function as follows: At low cell density LuxN and LuxQ autophosphorylate and convey phosphate through LuxU to LuxO. Phospho-LuxO indirectly represses *luxCDABE* expression. Therefore, no light is produced under these conditions. At high cell density, when LuxN and LuxQ interact with their autoinducer ligands they change from kinases to phosphatases that drain phosphate away from LuxO via LuxU. Unphosphorylated LuxO is inactive. LuxR binds the *luxCDABE* promoter and activates transcription. Therefore, under these conditions the bacteria produce light.

so *luxCDABE* is transcribed and the bacteria make light (35–37). A transcriptional activator called LuxR (not similar to *V. fischeri* LuxR) is absolutely required for the expression of *luxCDABE* (94, 142). A model for quorum sensing in *V. harveyi* is shown in Figure 6.

It is surprising that no components similar to the canonical LuxI/LuxR components of *V. fischeri* have ever been identified in *V. harveyi*. This is especially

noteworthy given how closely the two *Vibrio* species are related, and also given that the same target operons are regulated by quorum sensing in both cases. However, the opposite is not the case. A gene similar to *luxM* of *V. harveyi* has been found in *V. fischeri*. This gene is called *ainS* (45), and its product, AinS, directs the synthesis of a second *V. fischeri* HSL molecule (57, 78). The AinS protein does not have any homology to LuxI, but the mechanism of biosynthesis of the HSL appears to be the same (57). The role of the second autoinducer in *V. fischeri* remains a mystery, as inactivation of *ainS* has only a minor effect on bioluminescence (77, 78). Additionally, homologues of the *V. harveyi* LuxO and LuxU two-component proteins have been recently identified in *V. fischeri*. The genetic organization of the *luxOU* locus in *V. fischeri* is identical to that of *V. harveyi*, and furthermore, inactivation of *luxOU* in *V. fischeri* results in loss of repression of luminescence at low cell density. This phenotype closely resembles that determined for a *luxOU* mutant of *V. harveyi* (99). These results indicate that the quorum sensing system of *V. fischeri* could be much more complex than is currently thought. Furthermore, it appears that the *V. fischeri* system probably involves multiple signaling circuits similar to *V. harveyi*.

Interspecies Communication and the LuxS Family of AI-2 Synthases

The structure of the second *V. harveyi* autoinducer (AI-2) has not been identified. However, all evidence indicates that AI-2 is not an HSL (S. Schauder & B.L. Bassler, submitted). The gene that is required for production of AI-2 in *V. harveyi* has been identified (*luxS*), and LuxS has a role in the enzymatic synthesis of AI-2 (153). Highly conserved *luxS* homologues exist in a wide variety of both Gram-negative and Gram-positive bacteria. These *luxS*-containing bacteria produce AI-2 activity, and in every case tested, inactivation of *luxS* eliminates AI-2 production (5, 6, 153).

In contrast to the widespread nature of AI-2 and *luxS*, to date, only one other bacterium, *Vibrio parahaemolyticus*, which is very closely related to *V. harveyi*, has been shown to produce an AI-1 activity (6). These results led to the theory that, in *V. harveyi*, the AI-1 quorum sensing system is used for intraspecies cell-cell communication, whereas the AI-2 quorum sensing circuit is used for interspecies cell-cell communication (5, 6, 153). In natural habitats *V. harveyi* exists in mixed populations containing other species of bacteria. The ability to recognize and respond to multiple autoinducer signals from different origins could allow *V. harveyi* to monitor its own cell-population density and also the population density of other bacteria in the immediate environment. This capability could enable *V. harveyi* to gauge when it constitutes a majority or a minority of the total population. Furthermore, if *V. harveyi* could differentially respond to AI-1 and AI-2, this ability would enable it to alter its behavior appropriately based on when it exists alone versus when it exists in a consortium. A distinct role for each autoinducer in *V. harveyi* is supported by the recent finding that *V. harveyi* regulates multiple genes, in addition to *luxCDABE*, in response to AI-1 and AI-2. Furthermore, target

genes that are exclusively responsive to AI-1 and target genes that are only responsive to AI-2 have now been identified in *V. harveyi* (K. Mok, J. Henke, B.L. Bassler, unpublished data). These results indicate that *V. harveyi* could display an intricate series of behaviors in response to intra- and intercellular communication in the wild.

The species of bacteria that contain a gene homologous to *luxS* of *V. harveyi* were determined by database analysis using the translated *V. harveyi luxS* sequence. These bacteria include both Gram-negative and Gram-positive species. In every case the *luxS* genes were identified as parts of genome sequencing projects, and no function had been attributed to any LuxS prior to the *V. harveyi* findings and the subsequent analysis of the various *luxS* genes in AI-2 production (153). The species of bacteria that contain *luxS* genes include, but are not restricted to, *E. coli*, *S. typhimurium*, *Salmonella typhi*, *Salmonella paratyphi*, *Haemophilus influenzae*, *Helicobacter pylori*, *B. subtilis*, *Borrelia burgdorferi*, *Neisseria meningitidis*, *Yersinia pestis*, *Campylobacter jejuni*, *Vibrio cholerae*, *Mycobacterium tuberculosis*, *Enterococcus faecalis*, *S. pneumoniae*, *Streptococcus pyogenes*, *S. aureus*, *Clostridium perfringens*, *Clostridium difficile*, and *Klebsiella pneumoniae* (5, 153).

As *luxS* has only recently been discovered, there has not been sufficient time to identify many of the genes that are regulated by AI-2 in *luxS*-containing bacteria other than *V. harveyi*. A few pieces of information are currently available. AI-2 has been reported to induce the expression of the LEE pathogenicity island in *E. coli* O157. This island encodes a type III secretory apparatus that is required for virulence (148). A *Vibrio vulnificus luxS* mutant shows increased hemolysin production and delayed protease production. Furthermore, the LD_{50} for the *V. vulnificus luxS* mutant is 20-fold higher than that of wild-type *V. vulnificus* (75). In the Gram-positive bacterium *S. pyogenes* AI-2 is required for secretion of a cysteine protease virulence factor (92). Although preliminary, taken together, these results show that AI-2 acts as a signal in bacterial species besides *V. harveyi*. It will be interesting when the role of AI-2 is determined for many more bacteria that inhabit diverse habitats. Presumably, as in other quorum sensing systems, the use of AI-2 will have been appropriately adapted to augment survival in each specific locale.

QUORUM SENSING IN *MYXOCOCCUS XANTHUS:* A UNIQUE SYSTEM

Unlike other Gram-negative bacteria, *M. xanthus* displays quorum sensing behavior, but in this organism the phenomenon is not driven by an HSL autoinducer. *M. xanthus* is a soil bacterium that glides over solid surfaces and colonizes decaying plant material. These bacteria exhibit complex social behaviors in that they hunt for food in swarms, a behavior that allows the individual cells to take advantage of secreted hydrolytic enzymes produced by neighboring cells (26, 27). Upon starvation at high cell density, *M. xanthus* forms fruiting bodies. Bacteria inside the fruiting body undergo a developmental process that leads to spore formation.

Spore formation is partially controlled by a quorum sensing circuit, although nutrient limitation and a solid surface are also required for the differentiation process. A secreted signal called A-signal is required for density sensing in *M. xanthus* (53, 81). A-signal is a mixture of amino acids that are produced as a consequence of the action of extracellular proteases (79, 80, 127). Three loci are required to produce A-signal: *asgA*, *asgB*, and *asgC*. AsgA is a two-component histidine kinase, AsgB is a DNA binding transcriptional regulator, and AsgC is the major σ factor in *M. xanthus* (20, 126, 128). These proteins, along with other as yet unidentified proteins, are hypothesized to function in a pathway that activates the expression of genes specifying secreted proteases. These proteases in turn produce the mixture of amino acids that comprise the A-signal.

A-signal is sensed by another sensor kinase called SasS, which channels information, via phosphorylation, to the response regulator SasR (74, 173). Phospho-SasR interacts with the alternative σ factor σ^{54} to activate the downstream targets of this unique density-sensing system (49). A negative regulator called SasN must also be inactivated in order for *M. xanthus* to respond to A-signal. SasN is not homologous to any known protein, so its mechanism of function is not understood (172). A-signal dependent genes are hypothesized to encode structural and regulatory proteins required for spore development. In contrast to other bacteria, *M. xanthus* appears to have evolved a unique solution for the problem of how to assess cell-population density. Apparently, some facet(s) of the complex social lifestyle of this bacterium warrants the use of a distinct set of signaling molecules as well as an elaborate interconnected signaling network.

INTERGENERA AND INTERKINGDOM COMMUNICATION

Outlined above are only a few examples of the many known quorum sensing systems. Identification of novel quorum sensing circuits continues at an ever-increasing pace. Polymerase chain reaction (PCR) amplification of quorum sensing genes based on homology to known quorum sensing regulators is routinely used (156, 168). Furthermore, bioassays have been developed that greatly facilitate the cloning and identification of new quorum sensing regulators (96, 140, 168, 175). However, defining the regulons controlled by these new quorum sensing systems typically lags behind the isolation/identification of a new autoinducer/sensor pair. We know that at least 25 bacteria possess a LuxI/LuxR system, and at least that many bacteria possess LuxS and AI-2, but in most cases we do not understand what behaviors are controlled by these regulators.

In several cases it is clear that quorum sensing has a critical role in regulation of bacterial pathogenicity, and there is tremendous interest in designing and implementing novel antimicrobial strategies that expressly target quorum sensing. While synthetic strategies for the design of drugs that antagonize autoinduction are being developed by humans, it seems likely that eukaryotes susceptible to

infection by quorum sensing bacteria could have already evolved natural therapies designed to impede bacterial invasion/colonization by inhibiting quorum sensing mediated processes. Similarly, competing bacterial populations that are vying for colonization of a particular niche could attempt to thwart each other by targeting and inactivating one another's quorum sensing circuits.

Examples of eukaryotic interference with bacterial quorum sensing as well as bacterial interference with bacterial quorum sensing have been documented. In a study of eukaryotic interference with quorum sensing, the seaweed *Delisea pulchra* has been shown to possess natural agents that specifically counteract quorum sensing in the bacterium *Serratia liquefaciens*. It should be noted that this is a model system, and *S. liquefaciens* and *D. pulchra* do not actually encounter one other in nature. *S. liquefaciens* colonizes the seaweed by swarming over the surface of the plant leaves. Swarming motility in *S. liquefaciens* is controlled by an HSL quorum sensing system. The seaweed produces halogenated furanones and enones that are very closely related in structure to HSL autoinducers. The furanones and enones render *S. liquefaciens* incapable of swarming motility and therefore avirulent. These autoinducer antagonists have been shown to bind to the *V. fischeri* LuxR protein and inhibit it (93a). It is hypothesized that a similar mechanism of action is responsible for inhibiting pathogenicity in *S. liquefaciens* (29, 30, 46, 48). Although this is a model system, it is speculated that bacterial swarming over surfaces is likely to be critical for colonization and pathogenicity in marine/submerged environments, so this could be relevant for *D. pulchra* (47).

Above we describe one case of bacterial interference with bacterial quorum sensing in which the autoinducers from different *S. aureus* strains specifically inhibit each other's *agr* virulence systems (70, 95, 109). Another case of bacterial interference with bacterial quorum sensing has recently been reported involving *E. caratovora*. Both *B. subtilis* and *E. carotovora* are commensal soil organisms. *B. subtilis* produces an enzyme called AiiA that is homologous to zinc-binding metallohydrolases. This enzyme inactivates the *E. carotovora* HSL autoinducer. Although not certain, it is hypothesized that either AiiA hydrolyzes the amide bond that joins the acyl side chain to the homoserine lactone moiety of the autoinducer or AiiA hydrolyzes the ester bond in the lactone ring itself. Inactivation of the autoinducer by AiiA renders *E. carotovora* avirulent (24).

CONCLUSIONS AND FUTURE PERSPECTIVES

Bacteria occupying diverse niches have broadly adapted quorum sensing systems to optimize them for regulating a variety of activities. In every case quorum sensing confers on bacteria the ability to communicate and to alter behavior in response to the presence of other bacteria. Quorum sensing allows a population of individuals to coordinate global behavior and thus act as a multi-cellular unit. Although the study of quorum sensing is only at its beginning, we are now in a position to gain fundamental insight into how bacteria build community networks. We will learn

how quorum sensing has evolved to facilitate species-specific and interspecies cell-cell communication. We will learn how quorum sensing allows populations to act synergistically and how quorum sensing can be used to conquer competitors. We will learn about the assortment of signals that are employed by bacteria and about the biosynthesis of these signals as well as how the information encoded in these chemical signals is processed and transduced to control gene expression. Furthermore, novel antimicrobial strategies could be designed based on information garnered from studies of quorum sensing, which suggests that research on quourm sensing could have enormous practical applications.

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LITERATURE CITED

1. Ahmer BM, van Reeuwijk J, Timmers CD, Valentine PJ, Heffron F. 1998. *Salmonella typhimurium* encodes an SdiA homolog, a putative quorum sensor of the LuxR family, that regulates genes on the virulence plasmid. *J. Bacteriol.* 180:1185–93
2. Andersson RA, Eriksson AR, Heikinheimo R, Mae A, Pirhonen M, et al. 2000. Quorum sensing in the plant pathogen *Erwinia carotovora* subsp. *carotovora*: the role of *expR* (Ecc). *Mol. Plant Microbe Interact.* 13:384–93
3. Atkinson S, Throup JP, Stewart GS, Williams P. 1999. A hierarchical quorum-sensing system in *Yersinia pseudotuberculosis* is involved in the regulation of motility and clumping. *Mol. Microbiol.* 33:1267–77
4. Bainton NJ, Stead P, Chhabra SR, Bycroft BW, Salmond GP, et al. 1992. *N*-(3-oxohexanoyl)-L-homoserine lactone regulates carbapenem antibiotic production in *Erwinia carotovora*. *Biochem. J.* 288:997–1004
5. Bassler BL. 1999. How bacteria talk to each other: regulation of gene expression by quorum sensing. *Curr. Opin. Microbiol.* 2:582–87
6. Bassler BL, Greenberg EP, Stevens AM. 1997. Cross-species induction of luminescence in the quorum-sensing bacterium *Vibrio harveyi*. *J. Bacteriol.* 179:4043–45
7. Bassler BL, Wright M, Showalter RE, Silverman MR. 1993. Intercellular signalling in *Vibrio harveyi*: sequence and function of genes regulating expression of luminescence. *Mol. Microbiol.* 9:773–86
8. Bassler BL, Wright M, Silverman MR. 1994. Multiple signalling systems controlling expression of luminescence in *Vibrio harveyi*: sequence and function of genes encoding a second sensory pathway. *Mol. Microbiol.* 13:273–86
9. Bassler BL, Wright M, Silverman MR. 1994. Sequence and function of LuxO, a negative regulator of luminescence in *Vibrio harveyi*. *Mol. Microbiol.* 12:403–12
10. Beck von Bodman S, Farrand SK. 1995. Capsular polysaccharide biosynthesis and pathogenicity in *Erwinia stewartii* require induction by an *N*-acylhomoserine lactone autoinducer. *J. Bacteriol.* 177:5000–8
11. Beck von Bodman S, Hayman GT, Farrand SK. 1992. Opine catabolism and conjugal transfer of the nopaline Ti plasmid pTiC58 are coordinately regulated by a single repressor. *Proc. Natl. Acad. Sci. USA* 89:643–47
12. Brint JM, Ohman DE. 1995. Synthesis of multiple exoproducts in *Pseudomonas aeruginosa* is under the control of RhlR-RhlI, another set of regulators in strain

PAO1 with homology to the autoinducer-responsive LuxR-LuxI family. *J. Bacteriol.* 177:7155–63

13. Cao JG, Meighen EA. 1989. Purification and structural identification of an autoinducer for the luminescence system of *Vibrio harveyi*. *J. Biol. Chem.* 264:21670–76
14. Chernin LS, Winson MK, Thompson JM, Haran S, Bycroft BW, et al. 1998. Chitinolytic activity in *Chromobacterium violaceum*: substrate analysis and regulation by quorum sensing. *J. Bacteriol.* 180:4435–41
15. Choi SH, Greenberg EP. 1991. The C-terminal region of the *Vibrio fischeri* LuxR protein contains an inducer-independent *lux* gene activating domain. *Proc. Natl. Acad. Sci. USA* 88:11115–19
16. Choi SH, Greenberg EP. 1992. Genetic dissection of DNA binding and luminescence gene activation by the *Vibrio fischeri* LuxR protein. *J. Bacteriol.* 174:4064–69
17. Christie PJ. 1997. *Agrobacterium tumefaciens* T-complex transport apparatus: a paradigm for a new family of multifunctional transporters in eubacteria. *J. Bacteriol.* 179:3085–94
18. Cubo MT, Economou A, Murphy G, Johnston AW, Downie JA. 1992. Molecular characterization and regulation of the rhizosphere-expressed genes *rhiABCR* that can influence nodulation by *Rhizobium leguminosarum* biovar *viciae*. *J. Bacteriol.* 174:4026–35
19. Davies DG, Parsek MR, Pearson JP, Iglewski BH, Costerton JW, Greenberg EP. 1998. The involvement of cell-to-cell signals in the development of a bacterial biofilm. *Science* 280:295–98
20. Davis JM, Mayor J, Plamann L. 1995. A missense mutation in *rpoD* results in an A-signalling defect in *Myxococcus xanthus*. *Mol. Microbiol.* 18:943–52
21. Dawson M, Sia R. 1931. In vitro transformation of pneumococcal types I. A technique for inducing transformation of pneumococcal types in vitro. *J. Exp. Med.* 54:681–99
22. de Kievit TR, Iglewski BH. 2000. Bacterial quorum sensing in pathogenic relationships. *Infect. Immun.* 68:4839–49
23. Dessaux Y, Petit A, Tempe J. 1992. Opines in *Agrobacterium* biology. In *Molecular Signals in Plant-Microbe Interactions*, ed. DPS Verma, pp. 109–36. Boca Raton, FL: CRC
24. Dong YH, Xu JL, Li XZ, Zhang LH. 2000. AiiA, an enzyme that inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of *Erwinia carotovora*. *Proc. Natl. Acad. Sci. USA* 97:3526–31
25. Dunny GM, Brown BL, Clewell DB. 1978. Induced cell aggregation and mating in *Streptococcus faecalis*: evidence for a bacterial sex pheromone. *Proc. Natl. Acad. Sci. USA* 75:3479–83

25a. Dunney GM, Winans SC, eds. 1999. *Cell-Cell Signaling in Bacteria*. Washington, DC: ASM Press

26. Dworkin M. 1973. Cell-cell interactions in the myxobacteria. *Symp. Soc. Gen. Microbiol.* 23:125–47
27. Dworkin M, Kaiser D. 1985. Cell interactions in myxobacterial growth and development. *Science* 230:18–24
28. Eberhard A, Burlingame AL, Eberhard C, Kenyon GL, Nealson KH, Oppenheimer NJ. 1981. Structural identification of autoinducer of *Photobacterium fischeri* luciferase. *Biochemistry* 20:2444–49
29. Eberl L, Molin S, Givskov M. 1999. Surface motility of *Serratia liquefaciens* MG1. *J. Bacteriol.* 181:1703–12
30. Eberl L, Winson MK, Sternberg C, Stewart GS, Christiansen G, et al. 1996. Involvement of *N*-acyl-L-homoserine lactone autoinducers in controlling the multicellular behaviour of *Serratia liquefaciens*. *Mol. Microbiol.* 20:127–36
31. Engebrecht J, Nealson K, Silverman M. 1983. Bacterial bioluminescence: isolation and genetic analysis of functions from *Vibrio fischeri*. *Cell* 32:773–81

32. Engebrecht J, Silverman M. 1984. Identification of genes and gene products necessary for bacterial bioluminescence. *Proc. Natl. Acad. Sci. USA* 81:4154–58
33. Engebrecht J, Silverman M. 1987. Nucleotide sequence of the regulatory locus controlling expression of bacterial genes for bioluminescence. *Nucleic Acids Res.* 15:10455–67
34. Flavier AB, Ganova-Raeva LM, Schell MA, Denny TP. 1997. Hierarchical autoinduction in *Ralstonia solanacearum*: control of acyl-homoserine lactone production by a novel autoregulatory system responsive to 3-hydroxypalmitic acid methyl ester. *J. Bacteriol.* 179:7089–97
35. Freeman JA, Bassler BL. 1999. A genetic analysis of the function of LuxO, a two-component response regulator involved in quorum sensing in *Vibrio harveyi*. *Mol. Microbiol.* 31:665–77
36. Freeman JA, Bassler BL. 1999. Sequence and function of LuxU: a two-component phosphorelay protein that regulates quorum sensing in *Vibrio harveyi*. *J. Bacteriol.* 181:899–906
37. Freeman JA, Lilley BN, Bassler BL. 2000. A genetic analysis of the functions of LuxN: a two-component hybrid sensor kinase that regulates quorum sensing in *Vibrio harveyi*. *Mol. Microbiol.* 35:139–49
38. Fuqua C, Burbea M, Winans SC. 1995. Activity of the *Agrobacterium* Ti plasmid conjugal transfer regulator TraR is inhibited by the product of the *traM* gene. *J. Bacteriol.* 177:1367–73
39. Fuqua C, Eberhard A. 1999. Signal generation in autoinduction systems: synthesis of acylated homoserine lactones by LuxI-type proteins. See Dunney & Winans 1999, pp. 211–30
40. Fuqua C, Winans SC. 1996. Localization of OccR-activated and TraR-activated promoters that express two ABC-type permeases and the *traR* gene of Ti plasmid pTiR10. *Mol. Microbiol.* 20:1199–210
41. Fuqua C, Winans SC, Greenberg EP. 1996. Census and consensus in bacterial ecosystems: the LuxR-LuxI family of quorum-sensing transcriptional regulators. *Annu. Rev. Microbiol.* 50:727–51
42. Fuqua WC, Winans SC. 1994. A LuxR-LuxI type regulatory system activates *Agrobacterium* Ti plasmid conjugal transfer in the presence of a plant tumor metabolite. *J. Bacteriol.* 176:2796–806
43. Fuqua WC, Winans SC, Greenberg EP. 1994. Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators. *J. Bacteriol.* 176:269–75
44. Garcia-Lara J, Shang LH, Rothfield LI. 1996. An extracellular factor regulates expression of *sdiA*, a transcriptional activator of cell division genes in *Escherichia coli*. *J. Bacteriol.* 178:2742–48
45. Gilson L, Kuo A, Dunlap PV. 1995. AinS and a new family of autoinducer synthesis proteins. *J. Bacteriol.* 177:6946–51
46. Givskov M, de Nys R, Manefield M, Gram L, Maximilien R, et al. 1996. Eukaryotic interference with homoserine lactone-mediated prokaryotic signalling. *J. Bacteriol.* 178:6618–22
47. Givskov M, Eberl L, Molin S. 1997. Control of exoenzyme production, motility and cell differentiation in *Serratia liquefaciens*. *FEMS Microbiol. Lett.* 148:115–22
48. Givskov M, Ostling J, Eberl L, Lindum PW, Christensen AB, et al. 1998. Two separate regulatory systems participate in control of swarming motility of *Serratia liquefaciens* MG1. *J. Bacteriol.* 180:742–45
49. Gorski L, Gronewold T, Kaiser D. 2000. A sigma(54) activator protein necessary for spore differentiation within the fruiting body of *Myxococcus xanthus*. *J. Bacteriol.* 182:2438–44
50. Gray KM, Passador L, Iglewski BH, Greenberg EP. 1994. Interchangeability and specificity of components from the

quorum-sensing regulatory systems of *Vibrio fischeri* and *Pseudomonas aeruginosa*. *J. Bacteriol.* 176:3076–80

51. Gray KM, Pearson JP, Downie JA, Boboye BE, Greenberg EP. 1996. Cell-to-cell signaling in the symbiotic nitrogen-fixing bacterium *Rhizobium leguminosarum*: autoinduction of a stationary phase and rhizosphere-expressed genes. *J. Bacteriol.* 178:372–76
52. Grossman AD. 1995. Genetic networks controlling the initiation of sporulation and the development of genetic competence in *Bacillus subtilis*. *Annu. Rev. Genet.* 29:477–508
53. Hagen DC, Bretscher AP, Kaiser D. 1978. Synergism between morphogenetic mutants of *Myxococcus xanthus*. *Dev. Biol.* 64:284–96
54. Hahn J, Luttinger A, Dubnau D. 1996. Regulatory inputs for the synthesis of ComK, the competence transcription factor of *Bacillus subtilis*. *Mol. Microbiol.* 21:763–75
55. Hanzelka BL, Greenberg EP. 1995. Evidence that the N-terminal region of the *Vibrio fischeri* LuxR protein constitutes an autoinducer-binding domain. *J. Bacteriol.* 177:815–17
56. Hanzelka BL, Greenberg EP. 1996. Quorum sensing in *Vibrio fischeri*: evidence that *S*-adenosylmethionine is the amino acid substrate for autoinducer synthesis. *J. Bacteriol.* 178:5291–94
57. Hanzelka BL, Parsek MR, Val DL, Dunlap PV, Cronan JE Jr, Greenberg EP. 1999. Acylhomoserine lactone synthase activity of the *Vibrio fischeri* AinS protein. *J. Bacteriol.* 181:5766–70
58. Hassett DJ, Ma JF, Elkins JG, McDermott TR, Ochsner UA, et al. 1999. Quorum sensing in *Pseudomonas aeruginosa* controls expression of catalase and superoxide dismutase genes and mediates biofilm susceptibility to hydrogen peroxide. *Mol. Microbiol.* 34:1082–93
59. Havarstein LS, Coomaraswamy G, Morrison DA. 1995. An unmodified heptadecapeptide pheromone induces competence for genetic transformation in *Streptococcus pneumoniae*. *Proc. Natl. Acad. Sci. USA* 92:11140–44
60. Haverstein LS, Morrison DA. 1999. Quorum sensing and peptide pheromones in Streptococcal competence for genetic transformation. See Dunney & Winans 1999, pp. 9–26
61. Hinton JC, Sidebotham JM, Hyman LJ, Perombelon MC, Salmond GP. 1989. Isolation and characterisation of transposon-induced mutants of *Erwinia carotovora* subsp. *atroseptica* exhibiting reduced virulence. *Mol. Gen. Genet.* 217:141–48
62. Hoch JA. 1993. Regulation of the phosphorelay and the initiation of sporulation in *Bacillus subtilis*. *Annu. Rev. Microbiol.* 47:441–65
63. Hoch JA. 1995. Control of cellular development in sporulating bacteria by the phosphorelay two-component signal transduction system. See Hoch & Silhavy 1995, pp. 129–44
64. Hoch JA, Silhavy TJ. 1995. *Two-Component Signal Transduction*. Washington, DC: ASM Press
65. Hotchkiss RD. 1954. Cyclical behavior in pneumococcal growth and transformability occasioned by environmental changes. *Proc. Natl. Acad. Sci. USA* 40:49–55
66. Hui FM, Morrison DA. 1991. Genetic transformation in *Streptococcus pneumoniae*: nucleotide sequence analysis shows *comA*, a gene required for competence induction, to be a member of the bacterial ATP-dependent transport protein family. *J. Bacteriol.* 173:372–81
67. Hui FM, Zhou L, Morrison DA. 1995. Competence for genetic transformation in *Streptococcus pneumoniae*: organization of a regulatory locus with homology to two lactococcin A secretion genes. *Gene* 153:25–31
68. Hwang I, Li PL, Zhang L, Piper KR, Cook DM, et al. 1994. TraI, a LuxI homologue, is responsible for production of conjugation factor, the Ti plasmid

N-acylhomoserine lactone autoinducer. *Proc. Natl. Acad. Sci. USA* 91:4639–43

69. Janzon L, Arvidson S. 1990. The role of the delta-lysin gene (*hld*) in the regulation of virulence genes by the accessory gene regulator (*agr*) in *Staphylococcus aureus*. *EMBO J.* 9:1391–99
70. Ji G, Beavis R, Novick RP. 1997. Bacterial interference caused by autoinducing peptide variants. *Science* 276:2027–30
71. Ji G, Beavis RC, Novick RP. 1995. Cell density control of staphylococcal virulence mediated by an octapeptide pheromone. *Proc. Natl. Acad. Sci. USA* 92:12055–59
72. Jones S, Yu B, Bainton NJ, Birdsall M, Bycroft BW, et al. 1993. The *lux* autoinducer regulates the production of exoenzyme virulence determinants in *Erwinia carotovora* and *Pseudomonas aeruginosa*. *EMBO J.* 12:2477–82
73. Kaplan HB, Greenberg EP. 1985. Diffusion of autoinducer is involved in regulation of the *Vibrio fischeri* luminescence system. *J. Bacteriol.* 163:1210–14
74. Kaplan HB, Kuspa A, Kaiser D. 1991. Suppressors that permit A-signal-independent developmental gene expression in *Myxococcus* xanthus. *J. Bacteriol.* 173:1460–70
75. Kim SY, Lee SE, Kim YR, Kim JH, Ryu PY, et al. 2000. Virulence regulatory role of luxS quorum sensing system in *Vibrio vulmificus*. Presented at ASM General Meet.
76. Kleerebezem M, Quadri LE, Kuipers OP, de Vos WM. 1997. Quorum sensing by peptide pheromones and two-component signal-transduction systems in Gram-positive bacteria. *Mol. Microbiol.* 24:895–904
77. Kuo A, Blough NV, Dunlap PV. 1994. Multiple *N*-acyl-L-homoserine lactone autoinducers of luminescence in the marine symbiotic bacterium *Vibrio fischeri*. *J. Bacteriol.* 176:7558–65
78. Kuo A, Callahan SM, Dunlap PV. 1996. Modulation of luminescence operon expression by *N*-octanoyl-L-homoserine lactone in *ainS* mutants of *Vibrio fischeri*. *J. Bacteriol.* 178:971–76
79. Kuspa A, Plamann L, Kaiser D. 1992. Identification of heat-stable A-factor from *Myxococcus xanthus*. *J. Bacteriol.* 174:3319–26
80. Kuspa A, Plamann L, Kaiser D. 1992. A-signalling and the cell density requirement for *Myxococcus xanthus* development. *J. Bacteriol.* 174:7360–69
81. LaRossa R, Kuner J, Hagen D, Manoil C, Kaiser D. 1983. Developmental cell interactions of *Myxococcus xanthus*: analysis of mutants. *J. Bacteriol.* 153:1394–404
82. Latifi A, Foglino M, Tanaka K, Williams P, Lazdunski A. 1996. A hierarchical quorum-sensing cascade in *Pseudomonas aeruginosa* links the transcriptional activators LasR and RhIR (VsmR) to expression of the stationary-phase sigma factor RpoS. *Mol. Microbiol.* 21:1137–46
83. Lazazzera BA, Grossman AD. 1998. The ins and outs of peptide signaling. *Trends Microbiol.* 6:288–94
84. Lazazzera BA, Solomon JM, Grossman AD. 1997. An exported peptide functions intracellularly to contribute to cell density signaling in *B. subtilis*. *Cell* 89:917–25
85. Lee CY, Szittner RB, Miyamoto CM, Meighen EA. 1993. The gene convergent to *luxG* in *Vibrio fischeri* codes for a protein related in sequence to RibG and deoxycytidylate deaminase. *Biochim. Biophys. Acta* 1143:337–39
86. Lee MS, Morrison DA. 1999. Identification of a new regulator in *Streptococcus pneumoniae* linking quorum sensing to competence for genetic transformation. *J. Bacteriol.* 181:5004–16
87. Lewenza S, Conway B, Greenberg EP, Sokol PA. 1999. Quorum sensing in *Burkholderia cepacia*: identification of the LuxRI homologs CepRI. *J. Bacteriol.* 181:748–56
88. Lilley BN, Bassler BL. 2000. Regulation of quorum sensing in *Vibrio harveyi*

by LuxO and sigma-54. *Mol. Microbiol.* 36:940–54

89. Lina G, Jarraud S, Ji G, Greenland T, Pedraza A, et al. 1998. Transmembrane topology and histidine protein kinase activity of AgrC, the *agr* signal receptor in *Staphylococcus aureus*. *Mol. Microbiol.* 28:655–62
90. Lithgow JK, Wilkinson A, Hardman A, Rodelas B, Wisniewski-Dye F, et al. 2000. The regulatory locus *cinRI* in *Rhizobium leguminosarum* controls a network of quorum-sensing loci. *Mol. Microbiol.* 37:81–97
91. Luo ZQ, Qin Y, Farrand SK. 2000. The antiactivator TraM interferes with the autoinducer-dependent binding of TraR to DNA by interacting with the C-terminal region of the quorum-sensing activator. *J. Biol. Chem.* 275:7713–22
92. Lyon WR, Madden JC, Stein J, Caparon MG. 2001. Mutation of *luxS* affects growth and virulence factor expression in *Streptococcus pyogenes*. *Mol. Microbiol.* In press
93. Magnuson R, Solomon J, Grossman AD. 1994. Biochemical and genetic characterization of a competence pheromone from *B. subtilis*. *Cell* 77:207–16

93a. Manefield M, deNys R, Kumar N, Read R, Givskov M, et al. 1999. 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* 145:283–91

94. Martin M, Showalter R, Silverman M. 1989. Identification of a locus controlling expression of luminescence genes in *Vibrio harveyi*. *J. Bacteriol.* 171:2406–14
95. Mayville P, Ji G, Beavis R, Yang H, Goger M, et al. 1999. Structure-activity analysis of synthetic autoinducing thiolactone peptides from *Staphylococcus aureus* responsible for virulence. *Proc. Natl. Acad. Sci. USA* 96:1218–23
96. McClean KH, Winson MK, Fish L, Taylor A, Chhabra SR, et al. 1997. Quorum sensing and *Chromobacterium violaceum*: exploitation of violacein production and inhibition for the detection of *N*-acylhomoserine lactones. *Microbiology* 143:3703–11
97. Milton DL, Hardman A, Camara M, Chhabra SR, Bycroft BW, et al. 1997. Quorum sensing in *Vibrio anguillarum*: characterization of the *vanI/vanR* locus and identification of the autoinducer *N*-(3-oxodecanoyl)-L-homoserine lactone. *J. Bacteriol.* 179:3004–12
98. Miyamoto CM, Boylan M, Graham AF, Meighen EA. 1988. Organization of the *lux* structural genes of *Vibrio harveyi*. Expression under the T7 bacteriophage promoter, mRNA analysis, and nucleotide sequence of the *luxD* gene. *J. Biol. Chem.* 263:13393–99
99. Miyamoto CM, Lin YH, Meighen EA. 2000. Control of bioluminescence in *Vibrio fischeri* by the LuxO signal response regulator. *Mol. Microbiol.* 36:594–607
100. More MI, Finger LD, Stryker JL, Fuqua C, Eberhard A, Winans SC. 1996. Enzymatic synthesis of a quorum-sensing autoinducer through use of defined substrates. *Science* 272:1655–58
101. Morfeldt E, Janzon L, Arvidson S, Lofdahl S. 1988. Cloning of a chromosomal locus (*exp*) which regulates the expression of several exoprotein genes in *Staphylococcus aureus*. *Mol. Gen. Genet.* 211:435–40
102. Morfeldt E, Taylor D, von Gabain A, Arvidson S. 1995. Activation of alpha-toxin translation in *Staphylococcus aureus* by the trans-encoded antisense RNA, RNAIII. *EMBO J.* 14:4569–77
103. Nasser W, Bouillant ML, Salmond G, Reverchon S. 1998. Characterization of the *Erwinia chrysanthemi expI-expR* locus directing the synthesis of two *N*-acyl-homoserine lactone signal molecules. *Mol. Microbiol.* 29:1391–405

104. Nealson KH, Hastings JW. 1979. Bacterial bioluminescence: its control and ecological significance. *Microbiol. Rev.* 43:496–518
105. Novick RP. 1999. Regulation of pathogenicity in *Staphylococcus aureus* by a peptide-based density-sensing system. See Dunney & Winans 1999, pp. 129–46
106. Novick RP, Projan SJ, Kornblum J, Ross HF, Ji G, et al. 1995. The *agr* P2 operon: an autocatalytic sensory transduction system in *Staphylococcus aureus*. *Mol. Gen. Genet.* 248:446–58
107. Nyholm SV, McFall-Ngai MJ. 1998. Sampling the light-organ microenvironment of *Euprymna scolopes*: description of a population of host cells in association with the bacterial symbiont *Vibrio fischeri*. *Biol. Bull.* 195:89–97
108. Ochsner UA, Reiser J. 1995. Autoinducer-mediated regulation of rhamnolipid biosurfactant synthesis in *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. USA* 92:6424–28
109. Otto M, Sussmuth R, Vuong C, Jung G, Gotz F. 1999. Inhibition of virulence factor expression in *Staphylococcus aureus* by the *Staphylococcus epidermidis agr* pheromone and derivatives. *FEBS Lett.* 450:257–62
110. Parsek MR, Greenberg EP. 1999. Quorum sensing signals in development of *Pseudomonas aeruginosa* biofilms. *Methods Enzymol.* 310:43–55
111. Parsek MR, Greenberg EP. 2000. Acyl-homoserine lactone quorum sensing in gram-negative bacteria: a signaling mechanism involved in associations with higher organisms. *Proc. Natl. Acad. Sci. USA* 97:8789–93
112. Parsek MR, Val DL, Hanzelka BL, Cronan JE Jr, Greenberg EP. 1999. Acyl homoserine-lactone quorum-sensing signal generation. *Proc. Natl. Acad. Sci. USA* 96:4360–65
113. Passador L, Cook JM, Gambello MJ, Rust L, Iglewski BH. 1993. Expression of *Pseudomonas aeruginosa* virulence genes requires cell-to-cell communication. *Science* 260:1127–30
114. Pearson JP, Gray KM, Passador L, Tucker KD, Eberhard A, et al. 1994. Structure of the autoinducer required for expression of *Pseudomonas aeruginosa* virulence genes. *Proc. Natl. Acad. Sci. USA* 91:197–201
115. Pearson JP, Passador L, Iglewski BH, Greenberg EP. 1995. A second N-acylhomoserine lactone signal produced by *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. USA* 92:1490–94
116. Pearson JP, Pesci EC, Iglewski BH. 1997. Roles of *Pseudomonas aeruginosa las* and *rhl* quorum-sensing systems in control of elastase and rhamnolipid biosynthesis genes. *J. Bacteriol.* 179:5756–67
117. Peng HL, Novick RP, Kreiswirth B, Kornblum J, Schlievert P. 1988. Cloning, characterization, and sequencing of an accessory gene regulator (*agr*) in *Staphylococcus aureus*. *J. Bacteriol.* 170:4365–72
118. Perego M. 1997. A peptide export-import control circuit modulating bacterial development regulates protein phosphatases of the phosphorelay. *Proc. Natl. Acad. Sci. USA* 94:8612–17
119. Perego M, Hanstein C, Welsh KM, Djavakhishvili T, Glaser P, Hoch JA. 1994. Multiple protein-aspartate phosphatases provide a mechanism for the integration of diverse signals in the control of development in *B. subtilis*. *Cell* 79:1047–55
120. Pesci EC, Milbank JB, Pearson JP, Mc Knight S, Kende AS, et al. 1999. Quinolone signaling in the cell-to-cell communication system of *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. USA* 96: 11229–34
121. Pesci EC, Pearson JP, Seed PC, Iglewski BH. 1997. Regulation of *las* and *rhl* quorum sensing in *Pseudomonas aeruginosa*. *J. Bacteriol.* 179:3127–32
122. Pestova EV, Havarstein LS, Morrison DA. 1996. Regulation of competence for

genetic transformation in *Streptococcus pneumoniae* by an auto-induced peptide pheromone and a two-component regulatory system. *Mol. Microbiol.* 21:853–62

123. Pierson LS 3rd, Keppenne VD, Wood DW. 1994. Phenazine antibiotic biosynthesis in *Pseudomonas aureofaciens* 30-84 is regulated by PhzR in response to cell density. *J. Bacteriol.* 176:3966–74
124. Piper KR, Beck von Bodman S, Farrand SK. 1993. Conjugation factor of *Agrobacterium tumefaciens* regulates Ti plasmid transfer by autoinduction. *Nature* 362:448–50
125. Pirhonen M, Flego D, Heikinheimo R, Palva ET. 1993. A small diffusible signal molecule is responsible for the global control of virulence and exoenzyme production in the plant pathogen *Erwinia carotovora*. *EMBO J.* 12:2467–76
126. Plamann L, Davis JM, Cantwell B, Mayor J. 1994. Evidence that *asgB* encodes a DNA-binding protein essential for growth and development of *Myxococcus xanthus*. *J. Bacteriol.* 176:2013–20
127. Plamann L, Kuspa A, Kaiser D. 1992. Proteins that rescue A-signal-defective mutants of *Myxococcus xanthus*. *J. Bacteriol.* 174:3311–18
128. Plamann L, Li Y, Cantwell B, Mayor J. 1995. The *Myxococcus xanthus asgA* gene encodes a novel signal transduction protein required for multicellular development. *J. Bacteriol.* 177:2014–20
129. Pozzi G, Masala L, Iannelli F, Manganelli R, Havarstein LS, et al. 1996. Competence for genetic transformation in encapsulated strains of *Streptococcus pneumoniae*: two allelic variants of the peptide pheromone. *J. Bacteriol.* 178: 6087–90
130. Puskas A, Greenberg EP, Kaplan S, Schaefer AL. 1997. A quorum-sensing system in the free-living photosynthetic bacterium *Rhodobacter sphaeroides*. *J. Bacteriol.* 179:7530–37
131. Qin Y, Luo ZQ, Smyth AJ, Gao P, Beck Von Bodman S, Farrand SK. 2000. Quorum-sensing signal binding results in dimerization of TraR and its release from membranes into the cytoplasm. *EMBO J.* 19:5212–21
132. Reverchon S, Bouillant ML, Salmond G, Nasser W. 1998. Integration of the quorum-sensing system in the regulatory networks controlling virulence factor synthesis in *Erwinia chrysanthemi*. *Mol. Microbiol.* 29:1407–18
133. Rodelas B, Lithgow JK, Wisniewski-Dye F, Hardman A, Wilkinson A, et al. 1999. Analysis of quorum-sensing-dependent control of rhizosphere-expressed (*rhi*) genes in *Rhizobium leguminosarum* bv. *viciae. J. Bacteriol.* 181:3816–23
134. Rosemeyer V, Michiels J, Verreth C, Vanderleyden J. 1998. *luxI*- and *luxR*-homologous genes of *Rhizobium etli* CNPAF512 contribute to synthesis of autoinducer molecules and nodulation of *Phaseolus vulgaris*. *J. Bacteriol.* 180:815–21
135. Ruby EG. 1996. Lessons from a cooperative, bacterial-animal association: the *Vibrio fischeri-Euprymna scolopes* light organ symbiosis. *Annu. Rev. Microbiol.* 50:591–624
136. Ruby EG, McFall-Ngai MJ. 1992. A squid that glows in the night: development of an animal-bacterial mutualism. *J. Bacteriol.* 174:4865–70
137. Schaefer AL, Hanzelka BL, Eberhard A, Greenberg EP. 1996. Quorum sensing in *Vibrio fischeri*: probing autoinducer-LuxR interactions with autoinducer analogs. *J. Bacteriol.* 178:2897–901
138. Seed PC, Passador L, Iglewski BH. 1995. Activation of the *Pseudomonas aeruginosa lasI* gene by LasR and the *Pseudomonas* autoinducer PAI: an autoinduction regulatory hierarchy. *J. Bacteriol.* 177:654–59
139. Shadel GS, Young R, Baldwin TO. 1990. Use of regulated cell lysis in a lethal genetic selection in *Escherichia coli*: identification of the autoinducer-binding region

of the LuxR protein from *Vibrio fischeri* ATCC 7744. *J. Bacteriol.* 172:3980–87

140. Shaw PD, Ping G, Daly SL, Cha C, Cronan JE Jr, et al. 1997. Detecting and characterizing *N*-acyl-homoserine lactone signal molecules by thin-layer chromatography. *Proc. Natl. Acad. Sci. USA* 94:6036–41
141. Sheng J, Citovsky V. 1996. *Agrobacterium*-plant cell DNA transport: have virulence proteins, will travel. *Plant Cell* 8:1699–710
142. Showalter RE, Martin MO, Silverman MR. 1990. Cloning and nucleotide sequence of luxR, a regulatory gene controlling bioluminescence in *Vibrio harveyi*. *J. Bacteriol.* 172:2946–54
143. Singh PK, Schaefer AL, Parsek MR, Moninger TO, Welsh MJ, Greenberg EP. 2000. Quorum-sensing signals indicate that cystic fibrosis lungs are infected with bacterial biofilms. *Nature* 407:762–64
144. Sitnikov DM, Schineller JB, Baldwin TO. 1996. Control of cell division in *Escherichia coli*: regulation of transcription of *ftsQA* involves both *rpoS* and SdiA-mediated autoinduction. *Proc. Natl. Acad. Sci. USA* 93:336–41
145. Slock J, VanRiet D, Kolibachuk D, Greenberg EP. 1990. Critical regions of the *Vibrio fischeri* LuxR protein defined by mutational analysis. *J. Bacteriol.* 172:3974–79
146. Solomon JM, Lazazzera BA, Grossman AD. 1996. Purification and characterization of an extracellular peptide factor that affects two different developmental pathways in *Bacillus subtilis*. *Genes Dev.* 10:2014–24
147. Solomon JM, Magnuson R, Srivastava A, Grossman AD. 1995. Convergent sensing pathways mediate response to two extracellular competence factors in *Bacillus subtilis*. *Genes Dev.* 9:547–58
148. Sperandio V, Mellies JL, Nguyen W, Shin S, Kaper JB. 1999. Quorum sensing controls expression of the type III secretion gene transcription and protein secretion in enterohemorrhagic and enteropathogenic *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 96:15196–201
149. Stevens AM, Dolan KM, Greenberg EP. 1994. Synergistic binding of the *Vibrio fischeri* LuxR transcriptional activator domain and RNA polymerase to the *lux* promoter region. *Proc. Natl. Acad. Sci. USA* 91:12619–23
150. Stevens AM, Fujita N, Ishihama A, Greenberg EP. 1999. Involvement of the RNA polymerase alpha-subunit C-terminal domain in LuxR-dependent activation of the *Vibrio fischeri* luminescence genes. *J. Bacteriol.* 181:4704–7
151. Stevens AM, Greenberg EP. 1997. Quorum sensing in *Vibrio fischeri*: essential elements for activation of the luminescence genes. *J. Bacteriol.* 179:557–62
152. Surette MG, Bassler BL. 1998. Quorum sensing in *Escherichia coli* and *Salmonella typhimurium*. *Proc. Natl. Acad. Sci. USA* 95:7046–50
153. Surette MG, Miller MB, Bassler BL. 1999. Quorum sensing in *Escherichia coli*, *Salmonella typhimurium*, and *Vibrio harveyi*: a new family of genes responsible for autoinducer production. *Proc. Natl. Acad. Sci. USA* 96:1639–44
154. Swift S, Karlyshev AV, Fish L, Durant EL, Winson MK, et al. 1997. Quorum sensing in *Aeromonas hydrophila* and *Aeromonas salmonicida*: identification of the LuxRI homologs AhyRI and AsaRI and their cognate *N*-acylhomoserine lactone signal molecules. *J. Bacteriol.* 179:5271–81
155. Swift S, Lynch MJ, Fish L, Kirke DF, Tomas JM, et al. 1999. Quorum sensing-dependent regulation and blockade of exoprotease production in *Aeromonas hydrophila*. *Infect. Immun.* 67:5192–99
156. Swift S, Winson MK, Chan PF, Bainton NJ, Birdsall M, et al. 1993. A novel strategy for the isolation of *luxI* homologues: evidence for the widespread

distribution of a LuxR:LuxI superfamily in enteric bacteria. *Mol. Microbiol.* 10:511–20

157. Throup JP, Camara M, Briggs GS, Winson MK, Chhabra SR, et al. 1995. Characterisation of the *yenI/yenR* locus from *Yersinia enterocolitica* mediating the synthesis of two *N*-acylhomoserine lactone signal molecules. *Mol. Microbiol.* 17:345–56
158. Tomasz A, Hotchkiss RD. 1964. Regulation of the transformability of pneumococcal cultures by macromolecular cell products. *Proc. Natl. Acad. Sci. USA* 51:480–87
159. Turgay K, Hahn J, Burghoorn J, Dubnau D. 1998. Competence in *Bacillus subtilis* is controlled by regulated proteolysis of a transcription factor. *EMBO J.* 17:6730–38
160. Turgay K, Hamoen LW, Venema G, Dubnau D. 1997. Biochemical characterization of a molecular switch involving the heat shock protein ClpC, which controls the activity of ComK, the competence transcription factor of *Bacillus subtilis*. *Genes Dev.* 11:119–28
161. Val DL, Cronan JE Jr. 1998. In vivo evidence that *S*-adenosylmethionine and fatty acid synthesis intermediates are the substrates for the LuxI family of autoinducer synthases. *J. Bacteriol.* 180:2644–51
162. van Sinderen D, Luttinger A, Kong L, Dubnau D, Venema G, Hamoen L. 1995. *comK* encodes the competence transcription factor, the key regulatory protein for competence development in *Bacillus subtilis*. *Mol. Microbiol.* 15:455–62
163. Visick KL, McFall-Ngai MJ. 2000. An exclusive contract: specificity in the *Vibrio fischeri-Euprymna scolopes* partnership. *J. Bacteriol.* 182:1779–87
164. Welch M, Todd DE, Whitehead NA, McGowan SJ, Bycroft BW, Salmond GP. 2000. *N*-acyl homoserine lactone binding to the CarR receptor determines quorum-sensing specificity in *Erwinia*. *EMBO J.* 19:631–41
165. Whiteley M, Lee KM, Greenberg EP. 1999. Identification of genes controlled by quorum sensing in *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. USA* 96: 13904–9
166. Whiteley M, Parsek MR, Greenberg EP. 2000. Regulation of quorum sensing by RpoS in *Pseudomonas aeruginosa*. *J. Bacteriol.* 182:4356–60
167. Williams P, Bainton NJ, Swift S, Chhabra SR, Winson MK, et al. 1992. Small molecule-mediated density-dependent control of gene expression in prokaryotes: bioluminescence and the biosynthesis of carbapenem antibiotics. *FEMS Microbiol. Lett.* 79:161–67
168. Winson MK, Swift S, Fish L, Throup JP, Jorgensen F, et al. 1998. Construction and analysis of *luxCDABE*-based plasmid sensors for investigating *N*-acyl homoserine lactone-mediated quorum sensing. *FEMS Microbiol. Lett.* 163:185–92
169. Winzer K, Falconer C, Garber NC, Diggle SP, Camara M, Williams P. 2000. The *Pseudomonas aeruginosa* lectins PA-IL and PA-IIL are controlled by quorum sensing and by RpoS. *J. Bacteriol.* 182:6401–11
170. Withers HL, Nordstrom K. 1998. Quorum-sensing acts at initiation of chromosomal replication in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 95:15694–99
171. Wood DW, Gong F, Daykin MM, Williams P, Pierson LS 3rd. 1997. *N*-acylhomoserine lactone-mediated regulation of phenazine gene expression by *Pseudomonas aureofaciens* 30-84 in the wheat rhizosphere. *J. Bacteriol.* 179:7663–70
172. Xu D, Yang C, Kaplan HB. 1998. *Myxococcus xanthus sasN* encodes a regulator that prevents developmental gene expression during growth. *J. Bacteriol.* 180:6215–23
173. Yang C, Kaplan HB. 1997. *Myxococcus xanthus sasS* encodes a sensor histidine kinase required for early developmental gene expression. *J. Bacteriol.* 179:7759–67

174. Zhang L, Murphy PJ, Kerr A, Tate ME. 1993. *Agrobacterium* conjugation and gene regulation by N-acyl-L-homoserine lactones. *Nature* 362:446–48
175. Zhu J, Beaber JW, More MI, Fuqua C, Eberhard A, Winans SC. 1998. Analogs of the autoinducer 3-oxooctanoyl-homoserine lactone strongly inhibit activity of the TraR protein of *Agrobacterium tumefaciens*. *J. Bacteriol.* 180:5398–405
176. Zhu J, Winans SC. 1999. Autoinducer binding by the quorum-sensing regulator TraR increases affinity for target promoters in vitro and decreases TraR turnover rates in whole cells. *Proc. Natl. Acad. Sci. USA* 96:4832–37
177. Zhu J, Winans SC. 2001. The quorum-sensing regulator TraR requires autoinducer for protein folding, protease resistance and dimerization. *Proc. Natl. Acad. Sci. USA* 98:1507–12

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