Quorum Sensing within the Gut Ecosystem

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The successful modulation of phenotype is essential for the colonisation and proliferation of bacteria within the complex ecosystem of the gastrointestinal tract. To accomplish this, bacteria obtain and respond to information from the environment. One important parameter is the other bacteria present. The ability to correctly sense self, and also others, must therefore be advantageous for the control of mass action processes by the bacterial population. Within the gut ecosystem that may include processes involved in colonisation including those determining biofilm formation, pathogenicity, dispersal and DNA transfer. The ability to sense other bacteria may have important consequences for competitive and nutritional strategies controlling for example, entry into stationary phase, dispersal and the production of antimicrobial compounds. The ability to interfere with the signalling of bacteria will determine the fitness of the given organism to survive in the gut and may also have therapeutic potential. *Key words*: quorum sensing, colonisation, pathogenicity, gene transfer, dissemination.

INTRODUCTION

Cell-to-cell communication at high population density is often termed quorum sensing (see reviews 1–3). The principle involves the production of a signal molecule by a bacterium, which is released into the environment. As cell numbers increase so does the extracellular level of signal, until a threshold is reached. Gene activation, or in some cases de-repression or repression, may then occur via the activity of response regulator systems if other co-regulatory factors are satisfied. A model of how quorum sensing can regulate the expression of genes for high cell density growth in both Gram-negative and Gram-positive bacteria is presented in Figures 1 and 2. It is common for the gene encoding the signal generator to be part of the quorum sensing regulon, enabling a rapid build up of signal via a positive feedback loop. The first description of this system in the control of bioluminescence by *Vibrio fischeri* termed the process autoinduction and the signal molecule an autoinducer (4). Subsequently, these terms have been used to describe the process and the signals whether positive feedback occurs or not.

The study of communication systems in bacteria from many ecosystems has revealed a diversity of signals, signal generators, response regulators and regulated characters (3). A selection of chemically different molecules, illustrated in Figure 3, is utilized by bacteria for cell-to-cell signalling. In Gram-negative bacteria the best-characterized systems involve *N*-acylhomoserine lactone (acyl-HSL) signals, LuxI family signal synthases and LuxR family response regulators. It appears that Gram-positive bacteria prefer peptide signals, also termed peptide pheromones (5). These peptides are processed during secretion but may also be subject to complex post-translational modifications such as nisin in lactococci (6) or peptide thiolactones in staphylococci (7). Although there are, as yet, no chemically characterized examples of quorum sensing signal molecules common to both Gram-negative and Gram-positive bacteria, a gene termed *luxS* has been identified which has homologues in both types of bacteria (8). The LuxS protein appears to be a synthase responsible for the production of a novel class of quorum sensing signal molecules. Moreover, it is now becoming clear that a given organism may employ multiple quorum sensing signal molecules belonging to the same and or different chemical classes (9; L. E. N. Quadri, M. E. Stiles, M. Kleerebezem et al., unpublished data).

The study of cell-to-cell communication in gastrointestinal (GI) tract bacteria is not as advanced as it is for bacteria from other ecosystems. In this article we will describe how acyl-HSL and peptide signalling is involved in a number of well-characterized systems and then use some of these examples to illustrate where cell-to-cell signalling may be important to the commensal, dietary or probiotic microbes, and pathogenic members of the gut microbiota.

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SIGNAL GENERATION

Acyl-HSL biosynthesis involves a coupling of amino acid and fatty acid biosynthesis. The acyl groups of acyl-HSLs range from 4 to 14 carbons in length and can have various substitutions (see Fig. 3). The biochemistry of acyl-HSL formation by LuxI-type proteins has been studied *in vitro* (10–12) and requires *S*-adenosylmethionine (SAM) and an acylated acyl carrier protein (acyl-ACP) as substrates. A second family (LuxM) of acyl-HSL biosynthetic proteins has been identified (13, 14). Members of the LuxM family have no sequence homology with the LuxI family of acyl-HSL synthases, however, *in vitro* studies indicate that a similar mechanism is used, with the exception that the acylated coenzyme A can be used as a substrate almost as readily as acyl-ACP (15). A putative third type of acyl-HSL synthase (HdtS) in *Pseudomonas fluorescens* (B. Laue, Y. Jiang, S. R. Chhabra et al., unpublished data) awaits *in vitro* analysis.

In Gram-positive bacteria, peptide signals are made from post-translational processing of ribosomally synthesized peptides. A further common characteristic of the Gram-positive quorum sensing systems is the association of the processing, and often modification enzymes, and a dedicated ATP-binding cassette (ABC) export machinery for secretion of the signal (reviewed by 5). For example the inducing signals of the class II antimicrobial peptides (AMPs) are synthesized as pre-peptides with a leader sequence that is cleaved after the double-glycine motif in the peptide (16, 17). In fact these signals are similar to AMPs but are shorter and generally lack antimicrobial activity (Fig. 3). Gene clusters encoding AMPs also contain genes for secretion and cleavage of the leader sequence, and the immunity gene is located downstream of the AMP structural gene (reviewed by 18).

Many of the Gram-positive signalling peptides are post-translationally modified in addition to cleavage during the proteolytic secretion. The autoinducing peptides of staphy-

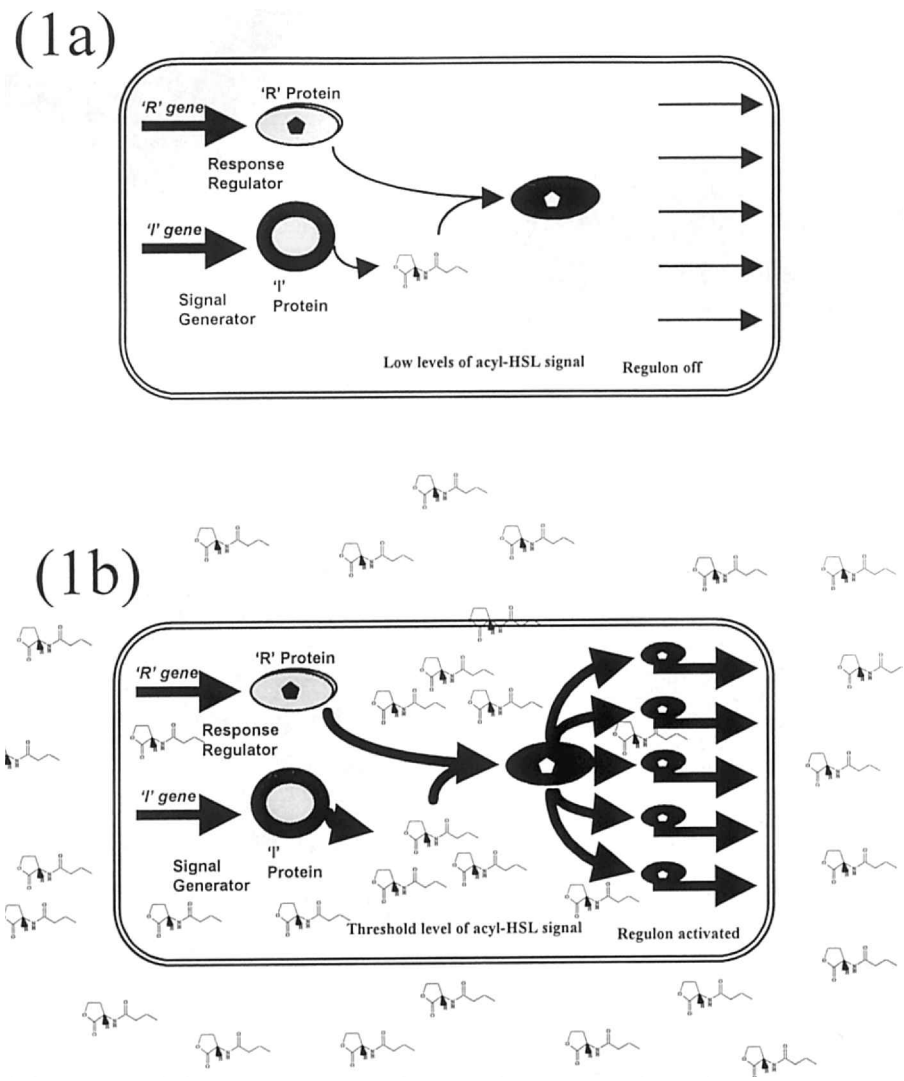

Fig. 1. Quorum sensing in Gram-negative bacteria: the activation of gene expression at high cell density. (a) and (b) show a model acyl-HSL system. (a) depicts the low cell density situation with a sub-threshold level of acyl-HSL (in this case C4-HSL) and no activation of the quorum sensing regulon. Throughout growth a low-level production of acyl-HSL occurs via the 'I' protein until the level of acyl-HSL reaches a concentration reflecting a threshold population cell density. (b) depicts the high cell density situation where the 'R' protein is activated and the quorum sensing regulon is expressed.

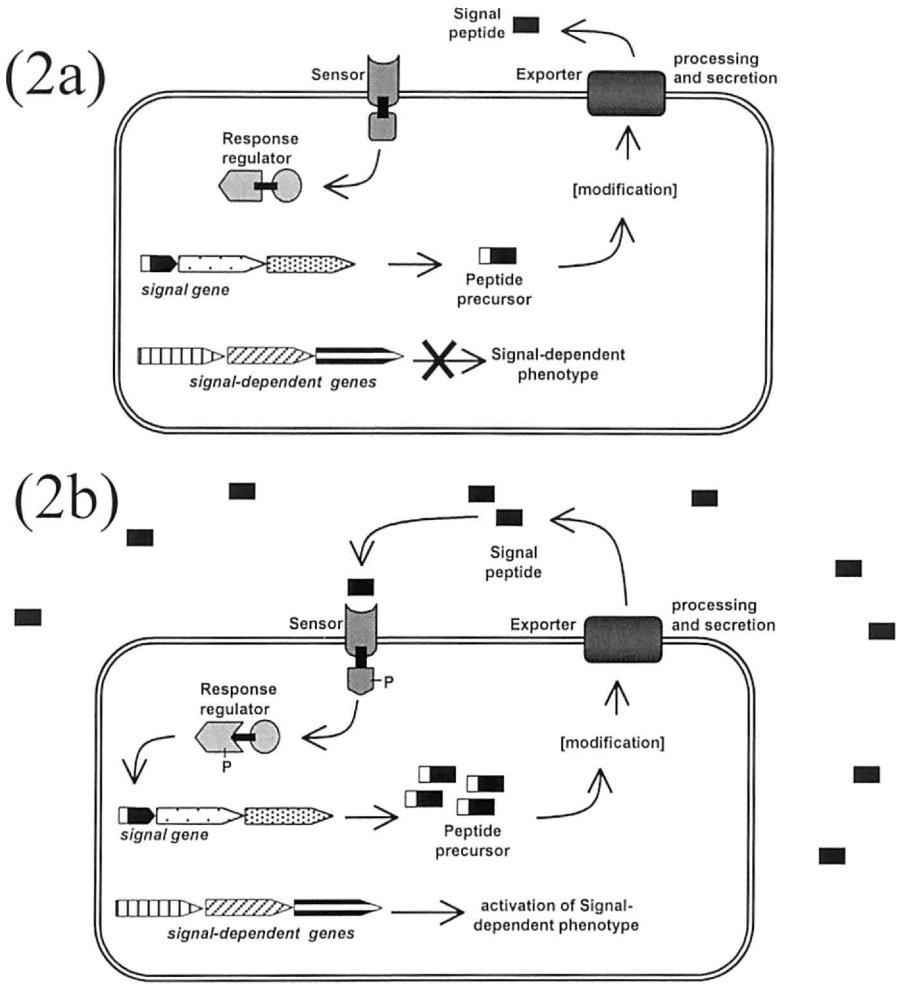

Fig. 2. Quorum sensing in Gram-positive bacteria: a model for peptide signalling through a two-component regulatory system. (a) illustrates the low level of signal peptide and inactivated signal-dependent regulon at low cell population density. In (b) a certain threshold concentration of signal is reached that triggers the signal transduction pathway and activates expression of signal-dependent genes.

lococci that control the synthesis of virulence and other extracellular proteins (reviewed by 19) have a cyclic thiolactone structure that is essential for biological activity (7). Lantibiotics (or class I AMPs) such as nisin and subtilin function as the AMP as well as the secreted signal, and they contain modifications such as dehydrated amino acids and lanthionine residues (Fig. 3) (20, 21). Recently, there have been indications to suggest that the enterococcal sex pheromones, which induce conjugative plasmid transfer (reviewed by 22), are processed at both the amino- and carboxy-terminal ends of the signal sequences from what appear to be surface lipoproteins of an as yet unknown function (23).

SIGNAL RESPONSE

Understanding signal biosynthesis may allow the directed design of inhibitors of quorum sensing. An understanding of how the response to the signal is mediated may also provide a therapeutic target. Members of the LuxR family mediate the response to the acyl-HSL signal. In the example of LuxR the acyl-HSL acts as an activating ligand to the transcriptional activation activity (reviewed by 24, 25). However, as more examples of acyl-HSL quorum sensing are investigated, so variations from the LuxR paradigm are described. LuxR itself is capable of acting as a repressor of *luxICDABE* expression at high acyl-HSL concentrations through a negative element within *luxD* (24). Furthermore, in *Pantoea stewartii* the LuxR homologue EsaR acts as a repressor of the genes required for exopolysaccharide (EPS) formation and so, a mucoidy appearance. In the wild-type organism EPS formation is cell density dependent (26). Signal generator (*esaI*) mutants do not produce EPS and are not virulent (27); however, the provision of exogenous acyl-HSL restores the mucoidy appearance. Conversely, *esaR* mutations constitutively produce EPS (26). De-repression, therefore, occurs upon interaction of EsaR with the acyl-HSL ligand and thus expression of this important virulence factor is targeted to populations at a high cell density. The DNA sequence of the LuxR binding site has been termed the *lux* box, and has features that are conserved at the binding sites of other LuxR type proteins (25). Additionally, LuxR requires specific interactions with the C-terminal domain of the η subunit of RNA polymerase to be made for transcriptional activation to occur (28).

The alternative signal generators of the LuxM family have a corresponding alternative group of response regulators. In *Vibrio harveyi* acyl-HSL signals have been shown to de-repress the *luxCDABEFG* operon via a phosphotransfer relay involving LuxN, LuxO and LuxU, allowing activation by LuxR to occur (29). It is noteworthy that the use of phosphorylation as a means of information transfer is not unique to quorum sensing, but is essential for the sensing of, and response, to the changing bacterial environment (30). The opportunity for cross talk between the quorum sensing phosphorelay and other phosphorelays therefore exists and has implications for the integration of many environmental signals.

The transfer of quorum sensing information via phosphotransfer has been studied most extensively in Gram-positive bacteria. The extracellular signal peptides of Gram-positive bacteria affect gene expression through signal transduction events that involve two components, the sensor and response-regulator proteins. The signal peptide is initially recognized by a surface membrane receptor located in the amino-terminus of a protein sensor that serves to regulate the activity of a histidine kinase domain in the carboxy-terminus at the membrane/cytoplasm interface. The sensor serves to modulate phosphorylation of a response-regulator that in turn modulates gene expression through effector molecules. A common theme among these

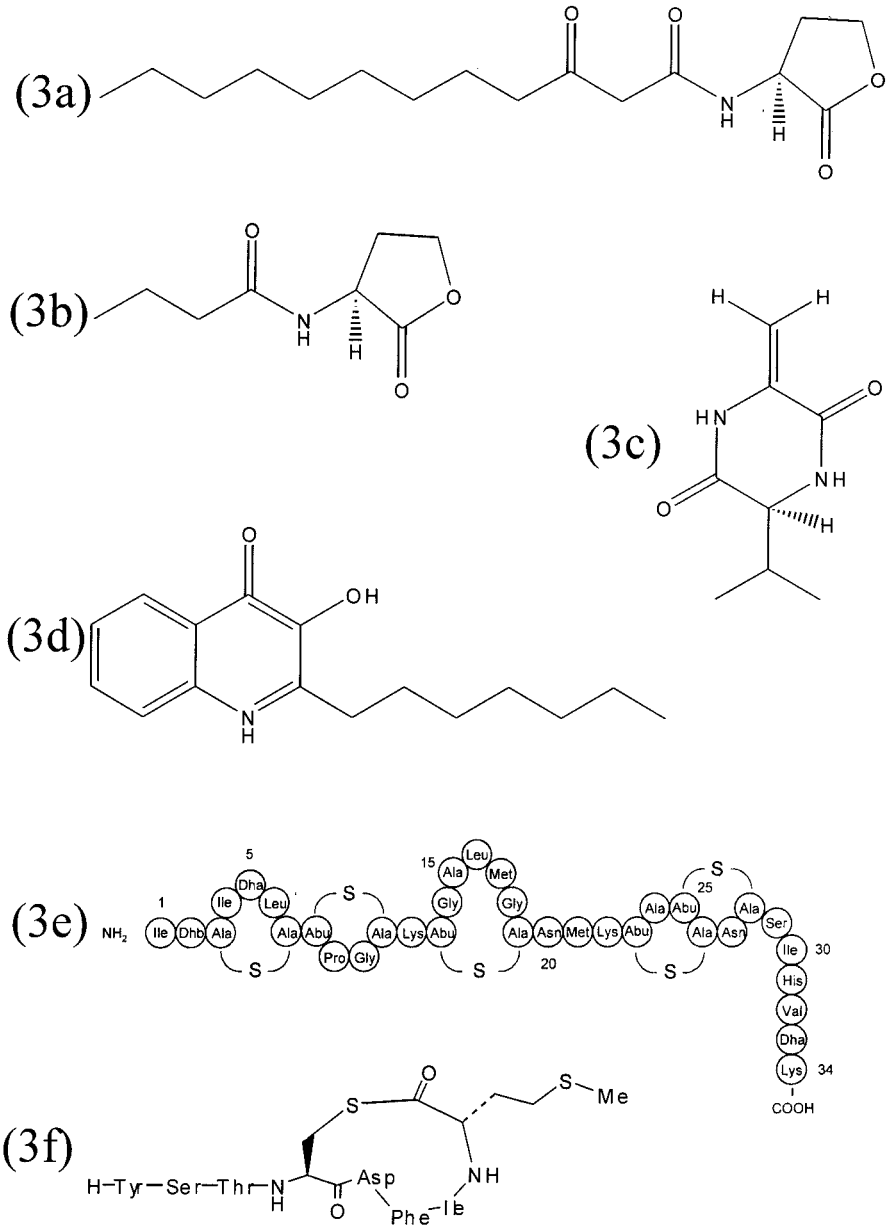

Fig. 3. The diversity of signal structures. The structure of signals identified in Gram-negative bacteria (a–d) and Gram-positive bacteria (e, f) are shown: Acylhomoserine lactones (a) *N*-(3-oxododecanoyl)-L-homoserine lactone; (b) *N*-(butanoyl)-L-homoserine lactone; a diketopiperazine; (c) cyclo(Δ-Ala-L-Val); (d) the *Pseudomonas* quinolone signal 2-heptyl-3-hydroxy-4-quinolone; (e) modified peptide nisin Z of *Lactococcus lactis*, (Ala-S-Ala: lanthionine, Abu-S-Ala: b-methyllanthionine, Dha: dehydroalanine, Dhb: dehydrobutyrine); and (f) group I *Staphylococcus aureus* cyclic peptide thiolactone.

systems is that the synthesis of the peptide signals appears to be autoregulated. Furthermore, the peptide structural gene and genes involved in export, and in some cases the sensor and response-regulator genes, are all transcribed together.

The regulation of nisin biosynthesis in *Lactococcus lactis* provides a well-documented example of a quorum sensing activated phosphotransfer system (reviewed by 21). The gene order in the nisin biosynthetic cluster is *nisABTCIPRKFEG*. The *nisKR* operon encodes the sensor and response regulator proteins and is constitutively transcribed from an independent promoter (31). NisR and NisK are the only components required for nisin-mediated signal transduction (31–33), where nisin acts as a signal to induce the transcription of two operons (*nisABTCIP* and *nisFEG*) that encode proteins for nisin biosynthesis and secretion, and nisin immunity (20). Preliminary evidence suggests that NisR binds to the *nisA* promoter that has been trimmed down to a 50 bp region (34).

In *Staphylococcus aureus* there is a further layer of complexity downstream of the response regulator that involves the production of an intracellular signal. The accessory gene regulator (*agr*) is a global regulatory locus in *S. aureus* that controls the expression of a range of virulence factors (reviewed by 19). A signal peptide is encoded by the *agr* locus (from the post-translational processing of AgrD by AgrB), and functions to activate *agr* transcription by signal transduction through sensor AgrC and response regulator AgrA. The nucleotide transcript, RNA III, that is also encoded in the *agr* locus, is the intracellular effector molecule for the quorum sensing activated *agr* response and is directly responsible for the up-regulation of secreted virulence factors and the down-regulation of surface proteins (35, 36). The activation of *agr* transcription is in conjunction with a transcription factor SarA and another locus *xpr* (37–39) that respond to other extracellular parameters. The integration of quorum sensing responses with the responses to other environmental conditions is a common occurence and will be detailed later.

In addition, extracellular factors may have direct intracellular effects. In the class II AMP system, a regulatory involvement of extracellular effectors such as pH and aeration is indicated, targeting class II AMP production to specific physiological conditions (40). It is feasible that the mechanism involved here acts via subtle alterations of the spatial structure of sensor-kinase proteins.

SIGNAL SPECIFICITY

The signal specificity of a number of LuxR homologs has been tested (41–45). In all cases a specific structure-activity relationship is seen. Furthermore, it is possible to block the action of the cognate acyl-HSL signal by the inclusion of antagonistic molecules in the growth medium. For example the C4-HSL activated protease activity of *Aeromonas hydrophila* can be blocked by the inclusion of longer chain ($>$ C8) acyl-HSLs in the growth medium (46). A similar phenomenon is seen in the blockade of C6-HSL induced pigment production in *cviI* mutants of *Chromobacterium violaceum* (42).

The recognition of the Gram-positive peptide signals by the corresponding sensor proteins is generally very specific. One exception has been noted within the class II AMPs, where the AMP of *Carnobacterium piscicola* could activate its own expression in a bacteriocin-negative culture, suggesting that the sensor protein recognizes the AMP as well as the signal pheromone (47; L. E. N. Quadri, M. E. Stiles, M. Kleerebezem et al., unpublished data). There are naturally occurring mechanisms by which quorum sensing may be blocked, for example in staphylococci there is inter-strain and inter-species variation in the structure of the quorum sensing signal peptide. Some *Staphylococcus* strains may, therefore, inhibit the *agr* response of other strains and thus *S. aureus* strains can be divided into several groups where the members within one group can induce RNA III transcription for each other, but inhibit RNA III transcription in the other groups (48). Another example is that of *Enterococcus faecalis* in which a range of pheromone signals are involved in the induction of conjugation between plasmid-containing donor cells and plasmid-free recipients. Each signal is encoded by, and specifically promotes the transfer of, one particular plasmid (49). The same plasmid also encodes a peptide that is secreted and acts as a competitive inhibitor of the pheromone (50). The inhibitor is believed to prevent self-induction of donors by endogenous pheromone when production is not completely switched off. It is also possible to construct functional hybrid sensor proteins. Chimeric genes encoding hybrid proteins of the N- and C-terminal domains of NisK and SpaK (specific for nisin and subtilin, respectively) were constructed whereby the specificity of the sensor could be inverted (21).

The use of bacterial signals as anti-bacterial pharmaceuticals, however, may not be viable. The long chain acyl-HSLs have biological effects upon host cells (51–53) and upon other bacteria, where they may activate genes for pathogenicity. Other compounds do exist that have a similar antagonistic effect upon quorum sensing activated phenotypes, for example the furanones secreted by the macroalgae *Delisea pulchra* (54) and the diketopiperazines (DKPs) produced by both bacteria and higher organisms (55). It is likely, therefore, that using the appropriate screening techniques a combinatorial chemistry approach may find candidate molecules for the blockade of quorum sensing as a pharmaceutical.

SIGNAL TRANSPORT

One of the fundamental aspects of quorum sensing is that the signal is able to move from inside the cell to outside the cell and then back inside the cell. Kaplan and Green-

berg (1985) showed that for 3-oxo-C6-HSL in *Vibrio fischeri*, the movement in and out was by free diffusion (56). The same free diffusion was assumed for subsequently discovered acyl-HSLs with different structures. *Pseudomonas aeruginosa* has two quorum sensing systems that utilize a short chain acyl-HSL, C4-HSL and a long chain acyl-HSL, 3-oxo-C12-HSL (57, 58). C4-HSL is able to move in and out of cells by free diffusion (59). 3-Oxo-C12-HSL, however, is concentrated 3 fold within the cell, possibly because of partitioning into bacterial membranes. Interestingly, although influx into the cell appears to be through diffusion, efflux from the cells is dependent upon an active MexAB-OprM pump (59). 3-Oxo-C12-HSL efflux is therefore dependent upon the proton motive force (PMF) of the cell and so the induction of the LasR/3-oxo-C12-HSL controlled regulon may involve a sensory input reflecting the energy status of the cell.

In Gram-positive bacteria, the sensor protein at the cell surface generally recognizes the extracellular signal peptide and signal transduction follows as described above. However, more examples of signalling peptides that function intracellularly to mediate cell-to-cell signalling are emerging that are not dependent on cell density. For example the enterococcal pheromones involved in conjugation are internalized by an oligopeptide permease and function by binding to intracellular regulatory proteins. A complex regulatory cascade follows that culminates in conjugative plasmid transfer (22). Similarly, the exported peptide CSF of *B. subtilis*, which is involved in DNA uptake and sporulation, is subsequently internalized again via an oligopeptide permease and functions intracellularly as detailed later (60).

INTEGRATED GENE REGULATION

Quorum-sensing control targets gene expression to high cell density populations for adaptation to growth at a high cell density. In many examples safeguards exist to prevent the premature induction of these genes for high cell density growth. In particular, early expression of those genes that affect interactions with higher organisms can adversely affect the bacterium. Identifying the molecular basis of these safeguards and understanding the conditions that relieve the safeguards is an important part of understanding the mass action processes of bacteria in complex environments like the gut. The value of this strategy can be seen in the context of the virulence of the plant pathogen *P. stewartii* (26). Mutants in *esaI* are unable to produce EPS and are therefore less virulent. The timing of exopolysaccharide expression is however crucial to the pathogenicity of *P. stewartii* and mutants in *esaR* produce EPS at low cell densities and are less virulent than the parent strain, presumably because the plant can respond earlier too and fight off the low level of bacteria.

The genetics of the higher levels of regulation are being unravelled in a number of species. For example, in the plant pathogens of the genus *Erwinia* repression of acyl-HSL biosynthesis and exoenzyme production is controlled by the *rsm* system. RsmA is a 6.8 kDa repressor protein (61) that is titrated by the regulatory RNA product of *rsmB* (62). Hence the level of free RsmA, determined by the ratio of RsmA to *rsmB* RNA, determines the extent of the repression. Mutations in *rsmA* over produce exoenzymes, whereas mutations in *rsmB* are repressed. Importantly, *rsmB* was originally identified as *aepH*, the transcription of which is induced in response to plant extracts (63). Homologues of *rsmA* have been identified in many bacteria and so it is likely that it is a generic method for gene regulation (64).

Other trans-acting factors repressing quorum sensing have been identified in another plant pathogen, *Agrobacterium tumefaciens*, where quorum sensing induces conjugal transfer of the tumour inducing (Ti) plasmid. Control focuses upon inhibition of the activity of the LuxR homolog, TraR, at low cell density. Two proteins are involved, TraM (65, 66) and TrlR/TraS (67, 68). TraM is thought to sequester TraR at low cell density as part of a negative feedback loop. *trlR/traS* is a dominant frameshift *traR* allele encoding a protein that lacks the DNA binding domain but is able to form inactive dimers. TrlR/TraS is induced in conditions of unfavourable nutrients, allowing TraR to accumulate only in conditions of abundant carbon and energy. Horizontal transfer of the Ti plasmid can then occur at high cell densities defined by acyl-HSL signalling (69).

In *Bacillus subtilis* quorum-sensing pathways and nutritional-sensing pathways are linked at the level of signal production to control entry into the stationary phase (reviewed by 70, 71). *B. subtilis* produces a number of signalling peptides that include the ComX pheromone and CSF (competence and sporulation factor) which both regulate the activity of a transcription factor ComA. ComX is a modified peptide pheromone that requires ComQ for production. ComP is the ComX-sensor protein that activates the response regulator protein ComA by phosphotransfer. The ComA response involves the development of genetic competence, the production of degradative enzymes, the production of the antibiotic surfactin and interestingly the predicted induction of a number of genes thought to be maximally expressed at the transition into stationary phase (71). Additionally, the production of a ComA-phosphatase enzyme, RapC, provides for a negative feedback regulation of ComA activity. CSF is the peptide signal derived from the product of the *phrC* gene, which is the second gene in a ComA activated operon with *rapC*. CSF is exported from the cell as pro-CSF and returns through the oligopeptide permease to interact with its intracellular targets. CSF complements the ComX signal by decreasing the rate of RapC dephosphorylation of

ComA. In conditions of starvation the sigma factor σ^H activates *phrC* transcription from a second promoter at the distal end of *rapC* (71, 72). The resultant starvation induced CSF production can then activate the ComA response at a lower cell density. At higher concentrations CSF is involved in a more drastic response to starvation, the induction of sporulation (reviewed in 70).

NON ACYL-HSL SIGNALLING IN GRAM-NEGATIVE BACTERIA

It is noteworthy that acyl-HSL signalling has not been described for well-studied Gram-negative bacteria such as *Escherichia coli* and *Salmonella* spp. The identity of *E. coli* promoters activated by conditioned media has been determined by a reporter transposon approach (73). The mutations were identified within genes involved in amino acid metabolism, however, the active component(s) of the conditioned medium was present in the aqueous fraction after extraction with an organic solvent and was not identified. Acyl-HSLs partition into the organic phase, from where purification and identification is relatively straightforward (74), however, purification and identification of signals partitioning into the aqueous phase has proven to be more technically challenging.

In *V. harveyi* acyl-HSL activation of bioluminescence via the alternative LuxM synthase family and LuxN, LuxO, LuxU and LuxR response regulation system has been characterized (29, 75). This work has also identified a second system, which involves a signal partitioning into the aqueous phase of organic solvent extracts (76). Again the structure of the signal is unknown but the synthase has been identifed and named LuxS (8). Homologues of the *luxS* gene are present in other Gram-negative and Gram-positive bacteria, including *E. coli* (8) where signal production and breakdown appears to be under strict control, giving a window of signalling opportunity (77). Analysis of the role of *luxS* in diarrhoeagenic *E. coli* strains has revealed activity in the enhancement of virulence factor expression (78). The role of quorum sensing in this example was interpreted as signalling the presence of other bacteria as is found at the site of pathogenesis, the intestine.

A further, as yet unidentified, *E. coli* signalling molecule that partitions into the aqueous phase after organic solvent extraction has been shown to inhibit the initiation of replication (79). A novel aspect to this inhibitory activity is that neither transcription nor translation is required for this signal to be effective.

Acyl-HSL signalling in *P. aeruginosa* has been the subject of much research (reviewed by 80). The identity of two new classes of signalling compounds from *P. aeruginosa* (also present in organic solvent extracts of the supernatants from other Gram-negative bacteria) has identified the concept of quorum sensing cross talk. 2-Heptyl-3-hydroxy-4-quinolone has been identified as an inducer of elastase production by *P. aeruginosa lasR* mutants (81), whereas diketopiperazines can act as activators or inhibitors in various quorum sensing reporting assays (55).

The diversity of different signalling compounds now apparent supports the assertion that most, if not all, bacteria use quorum sensing. Furthermore, we can also anticipate cross-talk between the microorganisms and their host. One example that may be beneficial is that of *Bacteroides thetaiotaomicron*, an abundant member of the normal microflora of the mouse and human small intestine. Colonisation of germ-free mice with *B. thetaiotaomicron* promotes the sustained expression of a fucosylated glycoconjugate by the enterocytes of the small intestine (82). The fucose is metabolized as an energy source by the bacterium, however, an isogenic strain carrying a transposon insert that disrupts the ability to utilize L-fucose could no longer induce the epithelial cells to produce the fucosylated carbohydrate. Recently a *B. thetaiotaomicron* regulatory protein, FucR, has been identified that acts in response to L-fucose. In the presence of L-fucose we see de-repression of the genes for fucose metabolism and repression of the genes inducing fucose production by the intestinal cells (83). It is noteworthy that colonisation with the η-fucosidase producing *Bifidobacterium infantis* was enhanced when it was introduced together into the mice with *B. thetaiotaomicron* (84). This research provides a glimpse of the possibilities of commensal microbe-host interactions in modifying the nutrient environment and thereby influencing the intestinal ecosystem. This type of model system should aid further studies of microbial and host interactions.

IS CELL-TO-CELL SIGNALLING IMPORTANT FOR THE COLONISATION OF THE HUMAN GUT IN HEALTHY INDIVIDUALS?

Quorum sensing systems have not yet been described for many of the species identified in the human GI tract. Nevertheless, there is evidence for the presence of acyl-HSLs in rumen samples (85). In those gut species that have been studied the results have little direct implications for colonisation. Of some relevance is the finding that quorum sensing is required for the formation of biofilms by both *P. aeruginosa* (86) and *A. hydrophila* (87). Linked with colonisation is the dispersal of bacteria from colonized areas. A role for quorum sensing in the induction of motility through liquids or along surfaces has now been shown in a number of organisms (88–91). In considering a role for quorum sensing in colonisation it is important to emphasize that the gut is not a sterile environment, it is already colonized and so in many respects is different to a laboratory monoculture or an initially sterile site of infection. We should therefore ask whether signalling has a role within the competitive and co-operative aspects of bacterial

colonisation of the intestine? And if so, can signalling be manipulated to prevent the colonisation of pathogens or promote the colonisation of probiotic species?

Peptide-pheromone induced cell density-dependent bacteriocin production has been described for several potential intestinal Gram-positive bacteria, such as *Lactobacillus plantarum* (92, 93), *Enterococcus faecium* (94), and *Ruminococcus gnavus* (A. Gomez, M. Ladire, M. Nardi et al., Abstract H-154, ASM, 99th General Meeting, Chicago, IL, 1999), and the probiotic *Lactobacillus johnsonii* La1 (C. Walker, M. Ventura, and R. D. Pridmore, Abstract H-61, ASM, 100th General Meeting, Los Angeles, CA, 2000). Furthermore, by designing oligonucleotide primers based on conserved sequences in the peptide pheromone-two-component signal transduction systems, novel histidine kinase-like genes were found in several intestinal microorganisms including *Lactobacillus johnsonii* and *Clostridium clostridiiforme*, as well as in DNA isolated from human faecal samples (J. Nakayama, A. D. L. Akkermans, W. M. de Vos et al., unpublished data). The ubiquitous nature of these (potential) two-component systems in Gram-positive intestinal bacteria suggests that they have a relevant *in vivo* function.

The potential quorum sensing system of bacteriocin ruminococcin A produced by *Ruminococcus gnavus* is presented in more detail because of its likely functionality *in vivo*. Ruminococcin A is a lantibiotic that is produced by *R. gnavus* FRE1, a strict anaerobe isolated from the human intestinal microflora (A. Gomez, M. Ladire, M. Nardi et al., Abstract H-154, ASM, 99th General Meeting, Chicago, IL, 1999). Three identical copies of a gene, *rumA*, encoding ruminococcin A are located downstream of two genes, *rumR* and *rumK*, whose deduced amino acid sequences is similar to response regulator and sensor proteins, respectively, of two-component signal transduction systems. The production of ruminococcin A is dependent on trypsin both *in vivo* in the intestine of gnotobiotic mice and *in vitro*. Trypsin is involved in the activation of both the operon that encodes the three *rumA* genes and the *rumRK* operon. It is proposed that trypsin is responsible for the processing of a putative extracellular inducer peptide that can function as the signal for the two-component signal transduction system. These results suggest the proposed quorum sensing control of bacteriocin production will only occur under circumstances perhaps specific to the preferred intestinal niche within the host.

IS CELL-TO-CELL SIGNALLING IMPORTANT FOR THE COLONISATION OF THE HUMAN GUT BY PATHOGENS?

There is evidence of a role for quorum sensing in the colonisation by pathogens where cell number, cell density and communication play an important role in virulence. The numerical data provide important parameters used in the study of quorum sensing and pathogenesis. It is relatively easy to determine the pathogenic dose using animal models and volunteer studies. Of course, this number (e.g. the LD_{50}) is composed of a sub-population that actually goes on to cause the disease, the rest may not survive the infection process or may pass through the gut. Hence, development of disease requires a minimum number of bacteria finding their site of action for disease to be caused. Often a period of proliferation is proposed during which bacteria multiply until sufficient numbers are present, for example for the induction of aggressins. The timing of aggressin expression may be crucial as the successful pathogen will have reached sufficient numbers to overcome the host defence prior to gene induction and that in the proliferation phase aggressin expression will be kept to a minimum to avoid activation of host defences. Examples of this concept in the link between pathogenicity and the timed regulation of EPS production by *P. stewartii* (26) and the controlled switching from an evasive phenotype to an aggressive phenotype by *S. aureus* have been mentioned earlier.

The interaction of pathogens with the commensal bacteria of the gut will also be important to understand, especially in the case of bacteria like *Escherichia coli*, where commensal and pathogenic strains exist. The study of the co-operation and/or competition between these bacteria will provide useful information, and if cell-to-cell signalling is involved we may be able to exploit this to tip the balance in favour of the commensal. For example, if a pathogen can signal to a barrier bacterium to disperse, allowing the pathogen access to the mucosal cells, blockage of that signal may provide an effective protection against the disease.

As cell density rises, so the nutritional demands made upon the environment will rise and eventually new food sources will be required. The bacterium may therefore expend significant amounts of energy liberating new nutrients during pathogenesis and will wish to protect this investment. Quorum sensing may be used to effect this by coupling antibiotic synthesis with exoenzyme production, as is seen in the cases of *E. carotovora*, *P. aeruginosa* and *C. violaceum* (reviewed by 74). As mentioned earlier, dispersal of the bacterium from colonies is an important step within the colonisation cycle and highlights the fact that colonisation is a dynamic process with many important events take place after the adhesion of the bacteria to the mucosal surface.

The biological effect of the signals upon the gut itself may also be significant as the immunomodulatory and cardivascular effects (51, 53) would most likely favour colonisation of the host by bacteria.

Table I

The consequences of quorum sensing in a natural environment.

	Signal loss	False signals	Signal interception
Causes	Diffusion; Chemical breakdown; Competitive removal	Signals from other species	Signal receptors of an unrelated organism
Negative effects	Energy loss to the cell; Less competitive phenotype expressed	Inappropriate gene expression	Attraction of competitors and predators; Initiation of host responses
Positive effects	Prevention of unnecessary gene expression; Rapid sensing of changes in the environment; Beneficial signal activities at other sites	Awareness of different species in the same niche	Co-ordination of consortial metabolism, Co-ordination of host and bacterial development, Modulation of host defences; Inhibition of competitors
Defences	Adapt the chemical properties of the signal; Regulate signal generation to occurs under specific environmental conditions; Decrease the threshold concentration of signal required to illicit a response	Increase receptor specificity; Increase threshold concentration of signal required to illicit a response, Integrate quorum sensing with overlapping signal pathways	Create community level response that are of general selective value; Develop new signals

QUORUM SENSING IN TIME AND SPACE

Bacteria within the gut are separated in space. We can envisage both inter-colony and intra-colony communication in processes that will be dependent upon the physical properties of the signal. In particular the stability of the signal, its diffusion and range, and partitioning between lipid membranes and the surrounding aqueous solutions are important factors. It is conceivable that gradients of a signal molecule could be used to attract or repel bacteria, such as signalling from cell associated bacteria to luminal bacteria. In the gut this is further complicated by the changing composition of the lumen contents with for example, differing pH between stomach and small intestine and the effect of emulsifying agents.

Bacteria are also separated in time and it may be that a signal has to be delivered not only to the right place in the gut, but also at the right time to elicit a response from the target bacterium. Indeed, we have already mentioned the case of the LuxS derived signal of *E. coli* that is degraded a short time after synthesis (77). Furthermore, it has now been shown that a Gram-positive bacterium (a *Bacillus*) can degrade the acyl-HSL signals of Gram-negative bacteria (95). The obvious benefit of this strategy suggests that analogous activities will also be present in other bacterial species and against other types of signal. Cell-to-cell signalling and quorum sensing have also been linked with the improved exit from dormancy of high-density populations of *Nitrosomonas europaea* (96) and *Micrococcus luteus* (97). This phenomenon may be of particular importance for bacteria coming into the gut that have entered a dormant state for survival. The consequences of quorum sensing in a natural environment (reviewed by 98) like the GI tract is summarized in Table I.

CONCLUDING REMARKS

For a better appreciation of how the microbial ecology of the gut influences the health of the whole organism it is important to understand how bacteria interact within the gut to influence the dynamics of colonisation and their subsequent activities. The evidence so far accumulated suggests that population cell density and cell-to-cell communication will be an important factor in the regulation of microbial activity within the high cell density bacterial population of the gut. The challenge now is to show that this is the case *in vivo* and then to use this information to help maintain a healthy gut microbiota.

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