MicroReview

Quorum sensing by peptide pheromones and two-component signal-transduction systems in Gram-positive bacteria

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Summary

Cell-density-dependent gene expression appears to be widely spread in bacteria. This quorum-sensing phenomenon has been well established in Gram-negative bacteria, where *N*-acyl homoserine lactones are the diffusible communication molecules that modulate cell-density-dependent phenotypes. Similarly, a variety of processes are known to be regulated in a cell-density- or growth-phase-dependent manner in Gram-positive bacteria. Examples of such quorum-sensing modes in Gram-positive bacteria are the development of genetic competence in *Bacillus subtilis* and *Streptococcus pneumoniae*, the virulence response in *Staphylococcus aureus*, and the production of antimicrobial peptides by several species of Gram-positive bacteria including lactic acid bacteria. Cell-density-dependent regulatory modes in these systems appear to follow a common theme, in which the signal molecule is a post-translationally processed peptide that is secreted by a dedicated ATP-binding-cassette exporter. This secreted peptide pheromone functions as the input signal for a specific sensor component of a two-component signal-transduction system. Moreover, genetic linkage of the common elements involved results in autoregulation of peptide-pheromone production.

Introduction

Several Gram-negative bacteria synthesize *N*-acyl homoserine lactones (AHLs), which function as diffusible communication-signal molecules and modulate discrete and diverse physiological processes in a cell-density- or growth-phase-dependent manner. The paradigm for AHL production is in the bioluminescence (*lux*) phenotype of *Vibrio fischeri* (later reclassified as *Photobacterium fischeri*), where the synthesis of the signal molecule *N*-(3-oxohexanoyl)-L-homoserine lactone (OHHL) requires the LuxI protein. The LuxR protein is presumed to bind to the OHHL molecule, thereby becoming the transcriptional activator that couples cell density to the expression of the bioluminescence genes. In many other Gram-negative species, similar autoinducing circuits have been identified that utilize similar components (AHL, LuxI and LuxR homologues) and play a role in cell-density-dependent regulation of cellular physiology (for a review see Salmond *et al.*, 1995).

Several processes in Gram-positive bacteria are known that are also regulated in a cell-density-dependent manner. Examples of these quorum-sensing systems are the development of genetic competence in *Bacillus subtilis* and *Streptococcus pneumoniae*, the virulence response in *Staphylococcus aureus*, and the production of antimicrobial peptides (AMPs) by several different species of Gram-positive bacteria. However, neither the cell-density-dependent regulatory processes mentioned here, nor, to our knowledge, any other regulatory processes in Gram-positive bacteria, involve an *N*-acyl homoserine lactone-like signal molecule in combination with a LuxI–LuxR related two-component system. An exception could be γ-butyrolactones (AHL structural homologues) that act as key regulatory molecules in controlling antibiotic production and differentiation in *Streptomyces* species. Despite the structural relatedness of the signal molecules involved, it seems unlikely that this regulatory mode involves the activity of LuxR or LuxI homologues (Horinouchi and Beppu, 1994). There appears to be an alternative common theme in cell-density-dependent regulatory modes of the Gram-positive bacteria mentioned, in that the signal molecule is a post-translationally processed (and sometimes
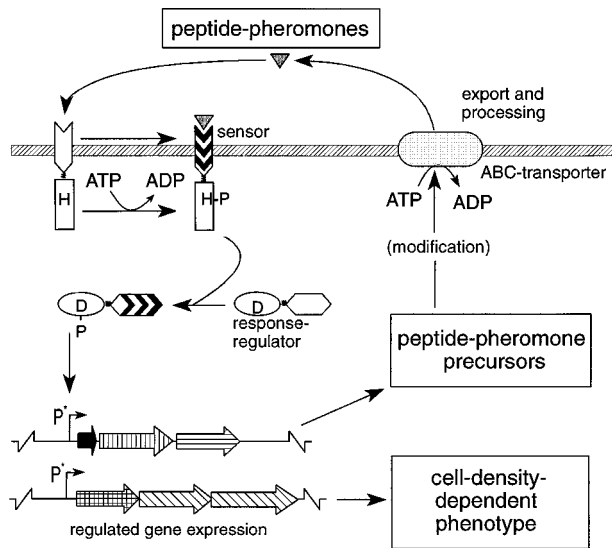
Fig. 1. Schematic representation of the model for the molecular modes involved in the quorum-sensing modules that are mediated by peptide pheromones and two-component regulatory systems in Gram-positive bacteria.

modified) secreted peptide. This secreted peptide phero-mone is recognized by the input domain of a typical sensor component of a two-component signal-transduction sys-tem (Fig. 1). Such two-component regulatory systems, consisting of a sensor and response-regulator protein, which use phosphorylation as a means to transfer infor-mation, form a major mechanism of signal transduction in bacteria and play a key role in many of the changes in cellular physiology that result from changes in the environ-ment (for reviews see Stock *et al.*, 1989; Parkinson and Kofoid, 1992). Another common feature in many of these quorum-sensing modes is the involvement of a dedicated ATP-binding cassette (ABC) exporter in the secretion of the peptide pheromone (Fig. 1). Furthermore, both the gene encoding the precursor of the peptide and the genes encoding the proteins involved in the two-component

regulatory system, and/or those involved in the secretion of the peptide pheromone, are transcriptionally linked, and the synthesis of the peptide pheromones appears to be an autoregulatory process.

The aim of this *MicroReview* is to give an overview of the characteristics of the components involved in these quorum-sensing modes and their relatedness. We will mainly focus on those quorum-sensing phenotypes for which the gen-etic organization and molecular characteristics of the com-ponents have recently been elucidated.

## Development of virulence and genetic competence

Relatively well-established phenotypes that are regulated in a cell-density-dependent manner are the development of natural genetic competence in both *B. subtilis* and *S. pneumoniae*, and the virulence phenotype of *S. aureus*.

Synthesis of a number of extracellular proteins that play an important role in the pathogenesis of *S. aureus* infec-tion is regulated in a cell-density-dependent manner. The regulation of this virulence phenotype is mediated by an RNA molecule (RNAIII; see below), whose expression level is controlled by the accessory gene regulator (*agr*) locus (Fig. 2) and by two further loci, *sar* and *xpr* (Mor-feldt *et al.*, 1996, and references therein). The *agr* locus contains two divergent transcription units. The larger of the two transcripts contains the *agr* operon encoding a sensor (*agrC*) and response-regulator (*agrA*) protein and two further open reading frames (ORFs), *agrB* and *agrD* (Fig. 2). The smaller transcript, RNA III, encodes the δ-lysin (*hld*; Fig. 2) and has a key regulatory role in the *agr*-response (Novick *et al.*, 1993; Morfeldt *et al.*, 1995). AgrC and AgrA can be regarded as being typical of the subgroup of Agr-like two-component regulatory systems (see below). These systems are characterized by sensor proteins that contain five to eight transmembrane seg-ments in their N-terminal domain, connected to a C-termi-nal domain that has features commonly found in histidine
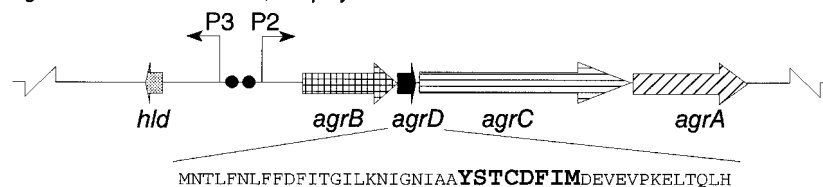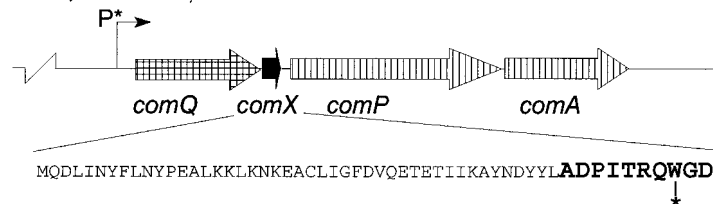
Fig. 2. Schematic representation of the genetic organization of the regulatory, *hld–agrBDCA* and *comQXPA* loci of *S. aureus* and *B. subtilis*, respectively. The structural gene encoding the peptide-pheromone precursor is indicated in black, the sensor proteins are indicated by horizontal hatching, and the response regulators are indicated by vertical hatching. The primary sequence of the peptide-pheromone precursor is indicated, with the amino acids that form the mature peptide pheromone indicated by larger, bold letters. The modification on the tryptophan residue in the *comX*-derived peptide is indicated by an asterisk. The direct repeats found in the P2 and P3 promoter regions are indicated by filled circles.

protein kinases. Likewise, the N-terminal domain of Agr-like response regulators share common sequences with other response regulators.

Recently, a modified octapeptide that was isolated from culture supernatants was shown to activate transcription from the *agr*-operon promoter (P2) as well as from the divergent RNAIII promoter (P3) in an *agrC*- and *agrA*-dependent manner (Ji *et al.*, 1995). The octapeptide is derived from an internal fragment of the *agrD* coding sequence (Fig. 2) and is thought to contain cyclic anhydride. Production of the *agrD*-derived peptide pheromone requires the *agrB* gene product, which might be involved in the post-translational modification of the primary *agrD* gene product (Ji *et al.*, 1995). These results suggest that AgrC acts as peptide-pheromone sensor and, via phosphorylation of AgrA, stimulates RNAIII production. However, a direct binding to DNA of AgrA has not yet been demonstrated. Moreover, a recent study suggests that binding of a second RNAIII regulatory protein (SarA) to the P2 and P3 promoter regions controls RNAIII production, and it remains unclear how AgrA influences this DNA binding of SarA (Morfeldt *et al.*, 1996). Earlier studies indicate that some environmental factors such as pH and glucose concentration have a strong influence on the *agr* expression level (Regassa *et al.*, 1992). It is unclear whether these environmental factors affect the *agr* expression level directly or via modulation of the efficiency of the AgrC-peptide-pheromone interaction.

The genetic organization and the factors involved in the development of genetic competence in *B. subtilis* strongly resemble the factors encoded by the *agr* locus of *S. aureus*. Natural genetic competence is the ability of cells to take up exogenous DNA. In a *B. subtilis* culture, grown under the appropriate conditions and to a high cell density, a subpopulation of cells differentiates to become competent. These competent cells are metabolically less active and produce a number of proteins that can bind and take up DNA independently of its nucleotide sequence (Dubnau, 1991). Production of this DNA-uptake machinery depends on the so-called 'competence transcription factor' ComK, which is subject to autoregulation. The transcription-enhancing activity of ComK is inhibited during exponential growth by direct interaction of ComK with MecA and MecB (ClpC). This inhibition can be relieved by a small protein ComS (Hahn *et al.*, 1996, and references therein), which is synthesized in response to a modified peptide pheromone that accumulates in the culture medium of *B. subtilis* strains when they are grown to high cell density (Magnuson *et al.*, 1994). The precursor of this peptide pheromone is encoded by the *comX* gene (Fig. 2). The nature of the post-translational modification remains unknown but it occurs on the unique tryptophan residue in the peptide and may involve the product of the *comQ* gene (Fig. 2) (Magnuson *et al.*, 1994). The cellular

response to the *comX*-derived peptide pheromone (synthesis of ComS) is mediated by a sensor (ComP) and a response-regulator (ComA) protein, encoded by *comP* and *comA*, respectively. These data suggest that ComP acts as a sensor for the *comX*-derived peptide pheromone and activates the *comS* transcription via signal transduction to ComA, which ultimately (via ComK) results in the development of genetic competence (for a review see Grossman, 1995). Although ComP and ComA appear to be their structural homologues, they do not share high primary-sequence similarity with AgrC and AgrA, respectively. The regulation of genetic competence is even further complicated by the presence of a second secreted peptide pheromone called the competence-stimulating factor (CSF). The action of CSF depends on the *spo0K* oligopeptide permease, suggesting that this peptide is taken up by the cell to stimulate the development of the competence phenotype, which could occur by modulation of the phosphorylation state of ComA (Solomon *et al.*, 1995; Grossman, 1995).

Remarkably, whereas the *comX*-derived peptide pheromone is the extreme C-terminal part of ComX, the *agrD*-derived peptide pheromone is an internal fragment of the primary *agrD* gene product (Fig. 2). Therefore, although both peptides are post-translationally modified 'subdomains' of small precursor proteins encoded within the regulatory locus, their processing routes are apparently different.

In contrast to the competence phenotype of *B. subtilis*, the competence phenotype of *S. pneumoniae* arises at early- or mid-logarithmic growth phase and persists only for a limited period of time. Recently, the competence pheromone in the culture supernatant of competent *S. pneumoniae* cells was found to be a 17-residue, unmodified peptide termed the competence-stimulating peptide (CSP), which could induce a similar short burst of competence at any point during the exponential growth of *S. pneumoniae*. The 41-residue precursor of CSP, encoded by the *comC* gene, has a characteristic N-terminal prosequence containing a Gly–Gly cleavage motif, which is commonly found in the precursor sequences of class II antimicrobial peptides (Håvarstein *et al.*, 1995; and see below). Interestingly, genes encoding an Agr-like two-component regulatory system (*comD* and *comE*, encoding a sensor and a response-regulator protein, respectively, that share significant homology with the *agrC* and *agrA* gene products) are present directly downstream of the *comC* gene (Fig. 3). The transcription of the *comCDE* genes depends directly on the *comE* gene product, indicating that the locus is autoregulatory (Pestova *et al.*, 1996). Based on the conservation of the *comCDE* locus among streptococcal strains, several *comC*-like genes have been identified and the corresponding peptide pheromones have been shown to induce competence in these
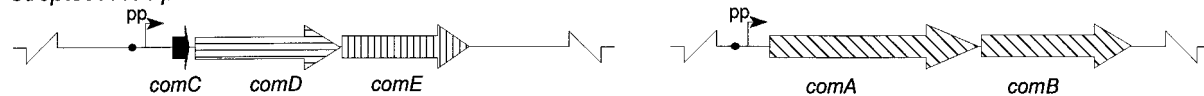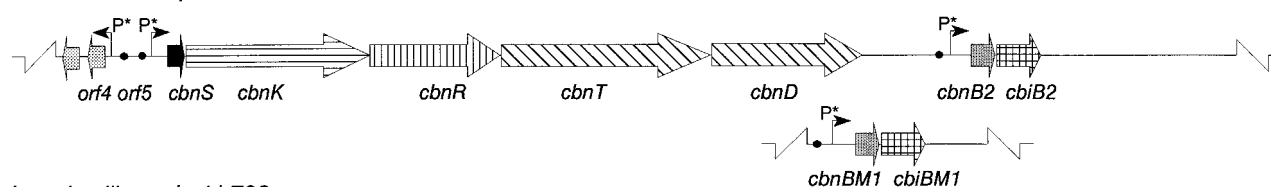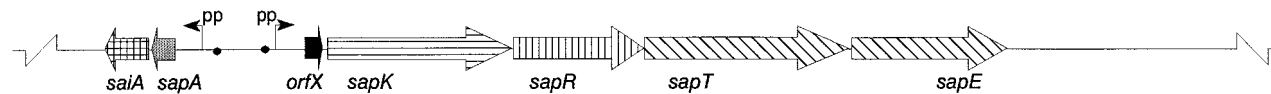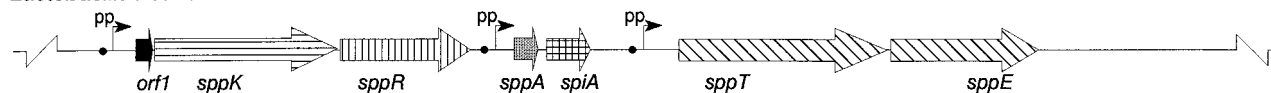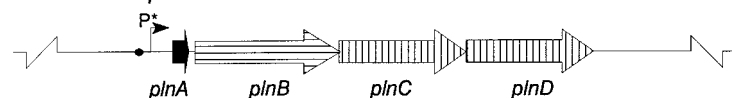
Fig. 3. Schematic representation of the gene clusters involved in competence development in *S. pneumoniae* (*comCDE* and *comAB* loci) and class II AMP production in various lactic acid bacteria: CbnB2 and CbnBM1 produced by *C. piscicola* LV17B, sakacin A by *L. sake* Lb706, sakacin P by *L. sake* Lb673, and plantaricins E,F,J,K and N by *L. plantarum* C11. Structural AMP-encoding genes are indicated in grey (filled arrows) and the corresponding immunity factor is encoded by the gene downstream of the structural genes (horizontal squares). Genes encoding (putative) peptide-pheromone precursors are indicated in black and are, in all cases, followed by the genes encoding the two-component system, sensor genes are indicated by horizontal hatching, and response-regulator genes are indicated by vertical hatching. Additional genes encoding proteins involved in transport of the AMPs and the peptide pheromones are indicated by forward hatching. The remaining genes are dotted and encode proteins with an unknown function. Regulated promoters that have been mapped are indicated by P*, and direct repeats within the (putative) promoter regions (pp) are indicated by filled circles. (Only the regulatory part of the plantaricin gene cluster found in *L. plantarum* C11 is shown; for the complete genetic organization of this cluster, the reader is refered to Diep *et al.* (1996). The gene cluster involved in sakacin P production is derived from *L. sake* strain Lb673 (Hühne *et al.*, 1996).)
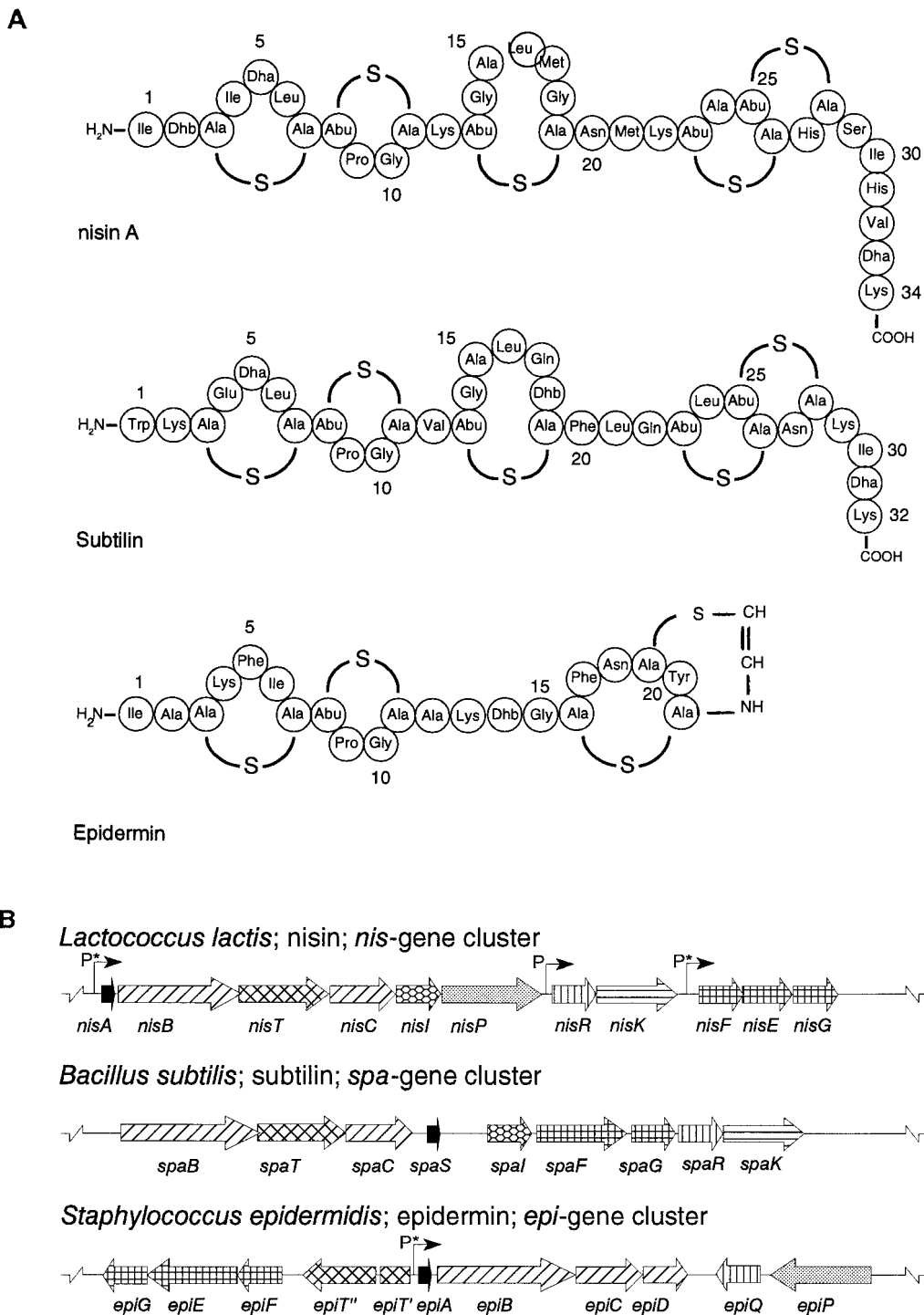
corresponding streptococcal strains, some of which were, until now, not regarded as naturally competent for transformation (Pozzi *et al.*, 1996; Håvarstein *et al.*, 1996). Taking advantage of the presence of two allelic forms of the *comC* and the corresponding *comD* genes found in two strains of *Streptococcus gordonii*, it was shown that the *comD* gene product really is the peptide-pheromone sensor (Håvarstein *et al.*, 1996). The secretion and processing of the primary *comC* gene product probably depends on the *comA* and *comB* gene products, which share significant homology with the ABC exporters and accessory proteins involved in the secretion of class II AMPs (Fig. 3) (Zhou *et al.*, 1995; and see below).

Regulation of lantibiotic biosynthesis

Lantibiotics or class I AMPs are heat-stable, post-translationally modified peptides; these modifications include dehydrated amino acids and typical (β-methyl)lanthionines (Fig. 4A). The genes required for lantibiotic biosynthesis, secretion and immunity are generally organized in a cluster (Fig. 4B) (for reviews see de Vos *et al.*, 1995; Siezen *et al.*, 1996).

Production of the lantibiotic nisin by *Lactococcus lactis* and subtilin by *B. subtilis* starts at early- to mid-logarithmic growth phase and increases to a maximal production level in the early stationary phase (Gutowski-Eckel *et al.*, 1994; de Ruyter *et al.*, 1996b). Interestingly, the nisin and the subtilin biosynthetic gene clusters (Fig. 4B) include genes encoding a sensor protein (*nisK* and *spaK*, respectively) and a response regulator (*nisR* and *spaR*, respectively) that have been shown to be essential for lantibiotic production (Siezen *et al.*, 1996, and references therein). Significant identity (26.4%) is found between the sensor proteins NisK and SpaK, which belong to the EnvZ-like subgroup of known sensor proteins. NisK and SpaK

A

nisin A

Subtilin

Epidermin

B

Lactococcus lactis; nisin; *nis*-gene cluster

nisA   nisB   nisT   nisC   nisI   nisP   nisR   nisK   nisF nisE nisG

Bacillus subtilis; subtilin; *spa*-gene cluster

spaB   spaT   spaC   spaS   spaI   spaF   spaG   spaR spaK

Staphylococcus epidermidis; epidermin; *epi*-gene cluster

epiG   epiE   epiF   epiT″   epiT′ epiA   epiB   epiC epiD   epiQ   epiP

Fig. 4. Lantibiotic biosynthesis.
A. Schematic representation of the structure of the lantibiotics nisin A, subtilin and epidermin.
B. Organization of lantibiotic gene clusters. The structural lantibiotic precursor genes are indicated in black, the sensor proteins are indicated by horizontal hatching and the response regulators are indicated by vertical hatching. Genes that play a role in the following processes are also indicated: precursor modification (tilting hatching), export (tilted squares), processing (dotted) or immunity (horizontal squares). Known promoters are indicated by P* for regulated promoters and P for non-regulated promoters. For further information regarding the organization and individual functions of the gene products, the reader is referred to Siezen *et al.* (1996).

have two predicted hydrophobic transmembrane helices in their N-terminal domain that flank a hydrophilic, presumably extracellular domain. The C-terminal domain of NisK contains the two conserved histidine kinase subdomains including the absolutely conserved histidine residue (Parkinson and Kofoid, 1992). Remarkable high identity is found between the response-regulator proteins NisR and SpaR (41.3%) which belong to the OmpR-like subgroup of response regulators.

In contrast to the nisin and subtilin gene clusters, the epidermin gene cluster (Fig. 4B) found in *Staphylococcus epidermidis* only contains a response-regulator-encoding gene (*epiQ*). Although EpiQ shares relatively low homology with either NisR or SpaR, the proteins with highest homology to EpiQ are members of the family of response regulators (for example PhoB of *Haemophilus influenzae* and *Escherichia coli*). In contrast to what is usually observed, their similarity to EpiQ is mainly limited to the C-terminal domain of EpiQ, and, moreover, the N-terminal domain of EpiQ lacks the conserved regions that contain the essential aspartate residues. Nevertheless, it has been shown that EpiQ controls epidermin production by transcriptional control of the *epiA* gene by binding to an inverted repeat which is located just upstream of the −35 region of the *epiA* promoter (Peschel *et al.*, 1993).

Nisin acts as signal molecule inducing its own synthesis

Recently, the nature of the environmental stimulus that activates the regulatory pathway involved in nisin biosynthesis by *L. lactis* has been elucidated. Introduction of a 4 bp deletion in the structural *nisA* gene (Δ*nisA*) of an *L. lactis* strain that normally produces nisin, resulted not only in loss of nisin production but also in abolition of Δ*nisA* transcription. An important finding leading to the insight that AMPs may have both antimicrobial and signaling activity, was the observation that transcription of Δ*nisA* could be restored by the addition of subinhibitory amounts of nisin (Fig. 4A) to the culture medium (Kuipers *et al.*, 1995). By fusion of a *nisA* promoter fragment to the promoterless reporter gene *gusA*, it was shown that the level of transcription of the *nisA* promoter is directly related to the amount of inducer that is added extracellularly (Kuipers *et al.*, 1995). Experiments using engineered nisin variants and nisin fragments indicated that the N-terminal residues within the fully modified nisin molecule play a more important role in induction as compared to the more C-terminally located residues (Kuipers *et al.*, 1995; Dodd *et al.*, 1996).

A terminator sequence directly downstream of the structural *nisA* gene probably allows *nisA* promoter-driven transcription of the downstream genes (*nisBTCIP*) by limited readthrough (Kuipers *et al.*, 1995, and references therein).

A second promoter within the nisin biosynthetic gene cluster upstream of *nisF* (Fig. 4B) drives transcription of the *nisFEG* genes and is also subject to nisin autoregulation (de Ruyter *et al.*, 1996a). In contrast, the third promoter located upstream of *nisRK* (Fig. 4B) drives constitutive expression of these two genes (de Ruyter *et al.*, 1996a). Disruption of *nisR* or *nisK* in a Δ*nisA* background abolishes activation of Δ*nisA* transcription by nisin (Kuipers *et al.*, 1995; M. Kleerebezem, unpublished). Furthermore, because expression of *nisRK* in other lactic acid bacteria allows nisin-inducible *nisA* promoter activity in these bacteria, NisR and NisK are the only components required for the signal-transduction pathway involved in nisin autoregulation (M. Kleerebezem *et al.*, submitted).

Taken together, these data indicate that, besides its function as an AMP, nisin also acts as a secreted signal molecule that induces the transcription of the genes involved in its own biosynthesis. Furthermore, the signal transduction for this process is mediated by a two-component regulatory system composed of NisK and NisR, indicating that nisin apparently acts as a peptide pheromone in this induction process.

Based on the substantial similarity between the nisin and subtilin biosynthesis systems, one can envisage a similar peptide-pheromone function for the mature subtilin molecule produced by *B. subtilis*. This assumption is supported by the finding that the production of subtilin is dependent on the presence of intact *spaR* and *spaK* genes (Siezen *et al.*, 1996, and references therein). The epidermin biosynthetic gene cluster appears to lack the sensor component of the two-component regulatory circuit completely (Fig. 4B; and see above). How the activity of the response regulator EpiQ of this system is regulated (if at all) remains unknown (Peschel *et al.*, 1993).

Regulation of class II antimicrobial peptide production

Class II AMPs (Klaenhammer, 1993) are small, heat-stable antimicrobial peptides that do not contain modified amino acid residues. Class II AMPs are synthesized as precursor peptides that contain an N-terminal extension that is removed during or shortly after secretion of the peptide. These pro-peptides of class II AMPs share a common feature in that two glycine residues precede the cleavage site (Gly−Gly motif). The current knowledge of the different class II AMPs and their corresponding secretion machinery and immunity (Klaenhammer, 1993) as well as their regulation of production (Nes *et al.*, 1996) have been recently reviewed.

Production of carnobacteriocin A, B2 and BM1 by *Carnobacterium piscicola* LV17 (Saucier *et al.*, 1995; Quadri *et al.*, 1994; 1997), several different putative plantaricins (PlnJK, PlnEF and PlnN) by *Lactobacillus plantarum* C11

Table 1. Amino acid sequences of (putative) peptide pheromones involved in the regulation of class II AMP production (see also Fig. 3) and competence development in *S. pneumoniae*.

Peptide	Amino acid sequence	PP	AMP
ComC	MKNTVKLEQFVALKEKDLQKIKGG $\triangledown$ EMRLSKFFRDFILQRKK	+	
CbnS	MKIKTITKKQLIQIKGG $\triangledown$ SKNSQIGKSTSSISKCVFSFFKKC	+	−
CbnB2	MNSVKELNVKEMKQLHGG $\triangledown$ VNYGNGVSCSKTKCSVNWGQAF-QERYTAGINSFVSGVASGAGSIGRRP	+	+
OrfX	MKLNYIEKKQLTNKQLKLIIGG $\triangledown$ TNRNYGKPNKDIGTCIWSGFRHC	?	?
Orf1	MMIFKKLSEKELQKINGG $\triangledown$ MAGNSSNFIHKIKQIFTHR	+	−
PlnA	MKIQIKGMKQLSNKEMQKIVGG $\triangledown$ KSSAYSLQMGATAIKQVKKLFKKWGW	+	−

The position of cleavage between the propeptide and the mature pheromone is indicated ($\triangledown$). The activities of the individual peptides CbnS, CbnB2, OrfX, Orf1 and PlnA as a peptide pheromone (PP) and/or antimicrobial peptide (AMP) are indicated.

(Diep *et al.*, 1995; 1996), and sakacin P by *Lactobacillus sake* LTH673 (Eijsink *et al.*, 1996), was lost upon inoculation of an overnight culture of a producing (Bac$^+$) strain into fresh culture medium at a level that is lower than a certain threshold inoculum size (varying from $<10^6$ colony-forming units (cfu) ml^{-1} to $<10^4$ cfu ml^{-1}). The obtained Bac$^-$ phenotype persisted during subsequent subcultivation in an inoculum-size-independent manner, but could be reversed by addition of a small volume (varying from 0.01% to 1% v/v) of cell-free Bac$^+$ culture supernatant, indicating the presence of an inducing factor in the Bac$^+$ culture supernatant. Thus the AMP production in these cells is regulated via a common mechanism that is apparent in inoculum-size-dependent loss of AMP production that can be reversed by addition of Bac$^+$ culture supernatant.

Significant sequence similarity is found among the components involved in the production of the AMPs by these bacteria (Fig. 3), and, based on this homology, the genes involved in the production of sakacin A by *L. sake* Lb706 could also represent a similar system (Axelsson and Holck, 1995). In all cases, the structural genes encoding the AMP are followed by a gene encoding the corresponding immunity protein, a genetic organization that is commonly found within the family of class II AMPs. Furthermore, all clusters contain genes encoding an ABC exporter and an accessory protein, which constitute a signal-sequence-independent class II AMP export- and processing machinery (Fig. 3). Moreover, two-component regulatory systems are encoded within these gene clusters, which are presumed to play a role in the regulation of AMP production (Fig. 3). All members of these two-component regulatory systems share significant amino acid sequence similarity

with both AgrC–AgrA and ComD–ComE (Nes *et al.*, 1996).

The Bac$^+$ phenotype inducing factors of *L. plantarum* C11 (Diep *et al.*, 1995) and *L. sake* LTH673 (Eijsink *et al.*, 1996) were purified from the Bac$^+$ culture supernatant and appeared to be AMP-like peptides (Table 1), which are encoded by a small ORF (*plnA* and *orf1*, respectively) preceding the gene encoding the sensor component (*plnB* and *sppK*, respectively) (Hühne *et al.*, 1996; Eijsink *et al.*, 1996; Diep *et al.*, 1995). A synthetic peptide based on the *cbnS* gene that encodes a AMP-like peptide (Table 1) and precedes the *cbnK* gene of *C. piscicola* LV17B, induced AMP production by *C. piscicola* LV17B (L. E. N. Quadri *et al.*, submitted). Furthermore, also the *sapK* gene of *L. sake* Lb706 is preceded by *orfX*, which encodes an AMP-like peptide (OrfX; Table 1), suggesting a similar inducer role for this gene product. However, no such data are available for *orfX*, and regulation of sakacin A production has not yet been reported. Overall, it is clear that both the genetic organization of the regulatory genes and the regulatory components found in class II AMP production strongly resemble the situation described for the regulation of competence development in *S. pneumoniae*.

The AMP-like characteristics of these peptide pheromones is supported by two findings: (i) they are produced as precursors with a typical double-glycine-type propeptide (Table 1), and (ii) similar to AMPs, they are predicted to be amphiphilic helices (Eijsink *et al.*, 1996). However, the inducer peptides differ from the AMPs in that they lack antimicrobial activity and are slightly shorter (AMPs are generally between 30 and 60 residues in length). It seems likely that the inducer peptides depend on the

same secretory machinery as AMPs. The inducer-peptide genes and the two-component genes are transcribed as one mRNA and are subject to inducer-peptide-mediated autoregulation (Diep *et al*., 1995; 1996; Eijsink *et al*., 1996; L. E. N. Quadri *et al*., submitted). These data suggest that the AMP-like inducer peptide can interact directly with the input domain of the corresponding sensor protein, which, via the response regulator, leads to activation of transcription of the target genes. However, only in the case of the *cbnS* product has it been shown that inducer activity is dependent on the sensor and response-regulator proteins (L. E. N. Quadri *et al*., submitted). The recognition of these peptide pheromones by the corresponding sensor proteins appears to be very specific (Diep *et al*., 1995; Pozzi *et al*., 1996). However, it appears that in *C. piscicola* LV17B, CbnB2 can also activate its own production in a Bac⁻ culture (Saucier *et al*., 1995; L. E. N. Quadri *et al*., submitted), suggesting that this AMP can also be recognized by the input domain of CbnK. Possibly, the CbnK protein recognizes the bacteriocin-like nature of CbnB2 and the *cbnS*-derived peptide, and could be the exception to the general rule.

The transcription of the genes involved in the production of class II AMPs was most extensively analysed in *C. piscicola* LV17B and *L. plantarum* C11 and several promoters were mapped within the gene clusters involved (indicated in Fig. 3). All of these promoters appear to be regulated, and the sequence alignment revealed the presence of a direct repeat of 9 or 10 nucleotides that are separated by 12–14 nucleotides and located 2–9 bp upstream of the −35 region (Fig. 3). These repeats could represent the binding site for the corresponding response regulator (Diep *et al*., 1996; L. E. N. Quadri *et al*., submitted). Remarkably, similar direct repeats can be identified upstream of the −35 regions of the putative promoters within the sakacin P and A gene clusters found in *L. sake* LTH673 and *L. sake* Lb706, respectively (Fig. 3). Furthermore, the P2 and P3 promoter within the *agr* locus contain similar direct repeats (Fig. 2) that have been suggested to be the SarA-binding sites (Morfeldt *et al*., 1996), and these repeats can also be found within the putative *comC* and *comA* promoters of *S. pneumoniae*. These repeats are conserved within one organism, suggesting that the corresponding promoters are under the same regulatory control (Nes *et al*., 1996).

In regulation of production of class II AMPs, the peptide pheromone involved is an AMP-like peptide that autoregulates its own synthesis as well as the synthesis of the corresponding AMP(s). However, the critical dependence on exogenous peptide pheromone to convert a Bac⁻ culture to a Bac⁺ culture at all stages of growth suggests that the level of production of endogenous peptide is insufficient to trigger AMP production at high cell density, which indicates a flaw in the cell-density dependency of these

systems. The observed lack of Bac⁻-to-Bac⁺ conversion in liquid media could be caused by the growth conditions used, which are obviously better than those in the natural environment. Interestingly, in this respect, it has been suggested that maximal AMP production is correlated to non-optimal growth conditions (de Vuyst *et al*., 1996). Alternatively, it seems possible that the constitutive expression of AMPs could, in fact, be regulated. It is noteworthy that fully functional regulatory systems that are already induced during early stages of growth can easily be overlooked. For example, the apparently unregulated production of sakacin A by *L. sake* Lb706 (Axelsson and Holck, 1995) might be regulated by the product of *orfX*. In contrast, bacteria that have been classified as non-AMP producing could in fact be AMP producers when grown under the appropriate conditions. This notion has important implications for the numerous AMP-screening studies of lactic acid bacteria that are used to identify AMPs useful for food preservation.

Concluding remarks

The regulation of cell-density-dependent phenotypes in Gram-positive bacteria can be represented in a common model (Fig. 1) in which a secreted proteinaceous molecule functions as communication signal. This peptide pheromone is likely to be directly sensed by the sensor component of a two-component regulatory system, which subsequently triggers both the production of the peptide pheromone itself and the corresponding cell-density-dependent phenotype. Secretion of the peptide pheromones is an active process that, in many cases, involves the activity of an ABC exporter (Fig. 1). In contrast, the AHL molecules involved in quorum-sensing modes found in Gram-negative bacteria appear to be freely diffusible across the outer and inner membranes. Furthermore, whereas internalization of the AHL molecule is required to trigger the corresponding response, the peptide-pheromone model does not include this step, because the sensor protein involved is located on the outer surface of the cytoplasmic membrane.

The cell-density-dependent regulatory model for Gram-positive bacteria implies that the peptide pheromone is produced at a low, constitutive level throughout exponential growth, reaching a certain threshold concentration at the end of this growth phase, which then triggers the signal-transduction pathway. However, the regulation of class II AMP production by several lactic acid bacteria and of competence development in streptococcal strains does not appear to fulfil this requirement, which might suggest the involvement of other (environmental) factors. Alternatively, it is possible that in response to suboptimal or changing physiological conditions the level of peptide-pheromone production is (temporarily) increased in order

to reach the threshold level, leading to what could be called a growth-conditions-dependent phenotype.

The peptide pheromones differ not only in the primary sequence of the peptide but also in the extent of post-translational modification, which may reflect specific stability requirements or, alternatively, could be required to ensure inducing specificity. Most of the peptide-pheromone-mediated regulatory systems depend on AgrC-like sensor components, which have N-terminal domains whose topology resembles that of transporter proteins and lacks any extensive surface-exposed domains. This could indicate that the peptide pheromones involved might be surface-active molecules (e.g. class II AMPs) that could adopt their membrane-associated conformation before interacting with the corresponding sensors. In contrast, NisK and SpaK, the sensors found in the lantibiotic-autoregulation systems, appear to belong to another class of sensor proteins (EnvZ-like) that contain one, relatively large, surface-exposed domain flanked by two transmembrane segments. This domain is generally believed to be the binding site for the corresponding inducing factor (Stock *et al*., 1989; Parkinson and Kofoid, 1992). Nevertheless, lantibiotics are also supposed to be surface-active molecules, which could indicate that NisK and SpaK sense these peptides predominantly via their transmembrane segments. Structure–function analyses of these sensor proteins should resolve these remaining questions.

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