Microbiology, Vol. 70, No. 1, 2001, pp. 1–9. Translated from Mikrobiologiya, Vol. 70, No. 1, 2001, pp. 5–14.

REVIEW PAPERS

Competence Pheromones in Bacteria

A. A. Prozorov

Vavilov Institute of General Genetics, Russian Academy of Sciences, ul. Gubkina 3, Moscow, 117809 Russia

Received January 24, 2000

Abstract—This article deals with general concepts concerning bacterial pheromones, including sex pheromones. The data on the structure and mode of action of competence pheromones in streptococci and bacilli are discussed, raising the question of the selective value of the process of genetic transformation for bacterial life activities and evolution.

PHEROMONES: GENERAL CONCEPTS

Pheromones are substances enabling distant communication among conspecifics. Pheromones can regulate individual behavior including reproduction-related activities. A classical example is provided by insect sex pheromones such as those of nocturnal saturnians (moths). Special organs located at the feather-like antennae of males can sense the female odor at a distance of several hundred meters. Pheromones released in social insects (bees, ants, etc.) can serve as a signal changing the status of all individuals in a community (a beehive, an anthill, etc.).

A large number of pheromones have been described in bacteria. They transmit signals from cell to cell, so that a bacterial culture becomes a simulacrum of a multicellular organism. Small peptides perform this function in a relatively large number of systems. However, pheromones also include aspartate, butyrolactone, *N*-acyl-L-homoserine lactone, branched-chain fatty acids, etc. [1]. As a rule, these substances represent relatively small molecules fairly resistant to environmental influences and active at very low concentrations (down to 10^{-11} M with *E. faecalis* sex pheromones: 1–5 pheromone molecules per cell are sufficient for the cell to sense and respond to these pheromones, which are octapeptides).

The mechanisms of sensing a pheromone signal and responding to it are partly related to the *quorum sensing* phenomenon. The term *quorum sensing* was coined in 1994 [2]. It signifies the cell's reception to environmental changes occurring when a bacterial culture reaches a certain threshold cell number and its response to these changes. The term was introduced in connection with the bioluminescence phenomenon in *V. fischeri*. This luminescent bacterium expresses its luminescence gene at the terminal stages of culture growth when the cell number is sufficiently high (at the late exponential and early stationary stages). However, an early stage culture can be made to emit light by the addition of the filtrate of a later stage culture, obviously indicative of a factor accumulating towards the end of culture growth. The authors of the article cited wrote that "certain bacterial behaviors can be performed efficiently only by a sufficiently large population of bacteria. We describe this minimum behavioral unit as a quorum of bacteria" [2, p. 273]. Occasionally, the term *density-sensing system* (a system estimating its own density) is used instead of the term *quorum sensing*. Quorum sensing can be based on monitoring various parameters including ion concentrations in the environment. A widespread example concerns the changes in pheromone concentrations (an increase in the cell concentration results in an increase in the concentration of a pheromone produced by the cells).

The simplest system used by a cell to sense a pheromone signal consists of two protein components, a sensor and a response regulator (Fig. 1). The sensor protein is composed of (i) a transmembrane receptor domain penetrating the cell surface layers and contacting the external space and (ii) a transmitting module embedded in the cytoplasm. A pheromone interacts with a receptor characterized by a sufficient affinity for it. The signal reaches the transmitting module and thus also the second component of this system, the response regulator protein. Signal transmission is accomplished by phosphorylating the regulator protein (involving the residues of the amino acids histidine and aspartate). The phosphorylated regulator interacts with the promoter of an operon that initiates the operation of previously silent genes (which may form a hierarchy; such a system is referred to as a regulon). This results in possibly drastic changes in the cell's metabolism. In essence, this is the description of the cell's response to a pheromone signal sensed by a receptor. A specific preformed two-component system corresponds to each of the potentially possible pheromones. Each receptor is tuned to a specific signal. For example, an *E. coli* cell possesses 40–50 such systems (reviewed in [3]). This creates the illusion that bacterial cells behave reasonably under changing environmental conditions. A large number of sensor proteins belong to the histidine kinase family that is capable of autophosphorylation [4]. Since pheromones cause whole chains of responses which are superimposed on one another, the situation in

0026-2617/01/7001-0001$25.00

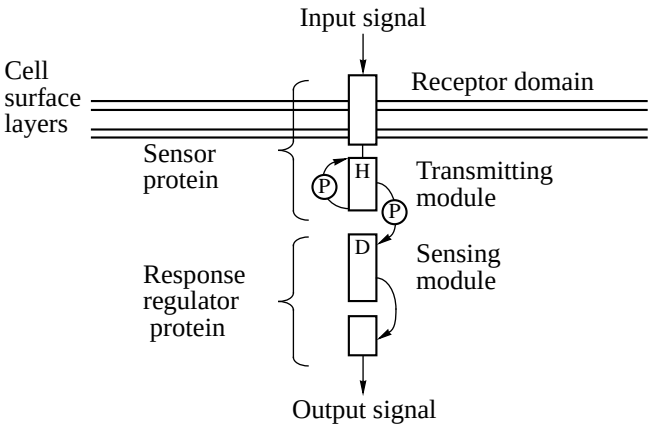

Fig. 1. Two-component signal-transmitting system (according to [3], modified). —℗→ Phosphorylation reaction; H and D, histidine and aspartate residues.

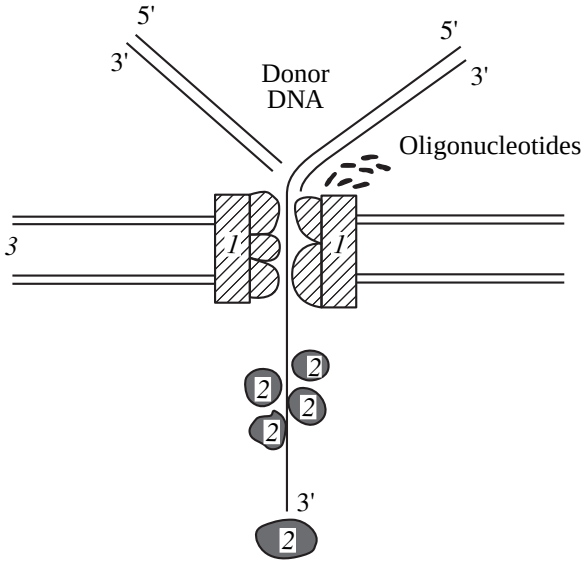

Fig. 2. Donor DNA processing during *Streptococcus* transformation (according to [56], modified). *1*, Proteins involved in the binding and cleavage of double-stranded DNA molecules, the formation of single-stranded molecules, and the DNA uptake; *2*, proteins binding to single-stranded DNA and recombination-related proteins; *3*, cell wall and cytoplasmic membrane

the cell is thereupon significantly more intricate than the above scheme. In addition, intracellular proteins can also serve as regulators. The mechanism of signal transmission is somewhat different from the classical prototype in the latter systems.

Bacterial pheromones perform quite diverse functions. The system regulating bioluminescence in *V. fischeri* was one of the first to be described. Pheromones can regulate antibiotic synthesis and the formation of aerial mycelium in actinomycetes; the production of extracellular protein virulence factors in staphylococci; the formation of the lantobiotic nisin in lactococci; the formation of fruiting bodies in myxococci, etc. [1, 4–7].

A self-contained group includes sex pheromones responsible for the conjugal transfer of plasmids in *E. faecalis* and Ti-plasmids in agrobacteria. This review concentrates on competence pheromones. Since the mechanism of action of these pheromones has been under study for a long time (about 40 years), it seems expedient to consider this research from a chronological perspective. From this example, it is evident that the success of a scientific initiative depends to a large extent on the advantages of the methods used by researchers.

COMPETENCE STATE IN BACTERIA

Competence in terms of genetic transformation usually implies the capacity of bacterial cells to bind and take up DNA. It seems reasonable to extend this notion to all subsequent stages including the recombination of the transforming DNA with the bacterial chromosome. In most systems, competence represents a transient state characteristic of cells in a growing population at the mid- and late-exponential growth stages. However, competence in some species is either related to the stationary growth stage (hemophilic bacteria) or omnipresent, irrespective of the growth stage (gonococci). The highest attainable competence level implies that either almost all cells in a population possess this property (this is the case with streptococci) or only a part of them (10–15% in bacilli). The DNA to be taken up can be added to the cells in the experiment or can appear in the medium as a result of the autolysis of a part of the cells of a growing culture (this causes spontaneous, or natural, transformation). This is the case with the growth of bacteria in a natural habitat. Importantly, some terminological confusion in the last decade has been caused by the fact that natural transformation is also construed to mean transformation accomplished by virtue of natural competence, not via DNA injection into cells based on artificial laboratory tools (protoplast transformation, electroporation, the calcium method of *E. coli* transformation, etc.). In my view, one should therefore retain the original term *spontaneous transformation* in relation to transformation via the DNA released from cells during autolysis.

The competence state is subject to regulation by a multitude of genes (at least 40 in bacilli). They are traditionally subdivided into the early and the late genes. The early genes include those *adjusting* a cell to competence acquisition. The late genes are responsible for DNA binding, uptake, and processing associated with DNA uptake (the cleavage of double-stranded molecules into small-size fragments, the formation of single-stranded DNA molecules as a result of destroying one of the original strands, and suction of single-stranded molecules into the cell using pilin proteins). The late genes also include those controlling DNA–chromosome recombination (Fig. 2). All of these genes

form a sophisticated hierarchy involving epistatic interactions. The mechanisms responsible for transformation may significantly differ in different bacterial species [8–11].

Following is the discussion of the mechanisms that turn on the competence state once a growing population reaches a sufficient density (the quorum density). These mechanisms are well understood in two groups of gram-positive bacteria, streptococci and bacilli. Competence pheromones perform a key function in these systems. It seems expedient to consider the peculiarities of this process in each of the two groups separately.

COMPETENCE PHEROMONES IN STREPTOCOCCI

All streptococci (*S. pneumoniae*, *S. gordonii*, *S. intermedius*, etc.) are closely related microorganisms that belong to one genus, although they may cause diverse diseases. The degree of DNA homology is sufficiently high between various species of streptococci and provides for interspecies crossing. A substance that subsequently proved to be a competence pheromone, was first described in these bacteria as far back as in the early 1960s. The first studies in this research direction were conducted by R. Pakula with the Challis strain of *S. gordonii* [12–15]. The main experiment was performed as follows. A culture at the early exponential (precompetent) growth stage was supplemented with the filtrate or with the redissolved ammonium sulfate precipitate of the medium taken from a midexponential stage (competent) culture. This resulted in the acquisition of the competence state by the immature culture. The substance producing this effect was termed the competence factor.

These articles and other works of the same period dealt with the properties of the competence factor. It was isolated by ammonium sulfate or ethanol precipitation. The competence factor was a protease-sensitive protein. Increasing the purification degree resulted in a decrease in its activity. The competence factor was relatively resistant to high temperatures. It was inactivated at 100°C by 20 and 100% in 10 and 90 min, respectively. Treating precompetent cells with competent cell-specific antibodies prevented the effect of the competence factor. Apparently, the antibodies bound to some cell surface receptor specific for the competence factor. The effect of the competence factor was also prevented by the addition of chloramphenicol to recipient cells, suggesting that competence induction required protein synthesis [16–18].

The substance stimulating competence acquisition in *S. pneumoniae* was detected two years later by A. Tomasz and R. Hotchkiss [19] and originally termed the activator. The activator's effect was first revealed in studies on cocultivating a competent and a precompetent culture in a V-shaped test tube separated by a cell-impermeable filter. The precompetent cells also attained the competence state. The properties of this activator were investigated in A. Tomasz' laboratory. The activator was partially purified and characterized. Based on its characteristics, it was considered a low molecular weight protein. It was trypsin-sensitive. Heating at 100°C inactivated it in 30 min. At a concentration of 0.1 μg/ml, the activator could induce the competence state in a population of 10^8 cells per ml. Chloramphenicol treatment rendered the cells activator-insensitive. Activator attachment on the cell surface implies the involvement of a preexistent protein distinct from the activator (referred to as the inhibitor). The isolated and purified inhibitor bound to the activator, suppressing its activity. Therefore, it was called the inhibitor [20–22].

The research on competence-stimulating substances in different *Streptococcus* species was continued in the 1970s and the 1980s. The main result obtained was that the effect of the competence factor and the activator involves the formation of a number of new proteins in the cell; the whole metabolism is restructured under the influence of these proteins (which form a cascade) [23–26].

This research was continued on a new instrumental level in the 1990s. It was (at least in part) completed in the studies conducted by D. Morrison's and L. Havarstein's groups [27]. The employment of a large number of new clinical isolates of streptococci and the goal-directed creation and mapping of mutations located in early competence genes significantly contributed to the successful completion of this research. The use of *the reporter gene* (the *lacZ* gene of *E. coli* responsible for β-galactoside synthesis) in combination with a promoter operating in the competence operon also proved a fruitful research strategy. This enabled the determination of the activity of this operon from the β-galactosidase amount synthesized in the system. The portions of the *Streptococcus* genome incorporating the competence genes were sequenced; the methods of purification of the activator and the competence factor were improved.

The in vitro synthesis of peptides identical to natural pheromones was also an efficient strategy. The terms *activator* and *competence factor* were substituted in the last decade by the abbreviation CSP (competence stimulating factor) or the term *pheromone* (used hereafter in this article).

The main results of the recent studies on *Streptococcus* pheromones can be summed up as follows. These pheromones are small cationic peptides. For instance, the *S. pneumoniae* pheromone is a sequence of 17 amino acid residues, H-Glu-Met-Arg-Leu-Ser-Lys-Phe-Phe-Arg-Asp-Phe-Ile-Leu-Gln-Arg-Lys-Lys-OH [28]; the pheromones of other streptococci consist of 14–23 residues. This accounts for their comparatively high thermostability revealed as early as in the 1960s. The activity of these pheromones can be extremely high. The competence state in *S. pneumoniae* can be

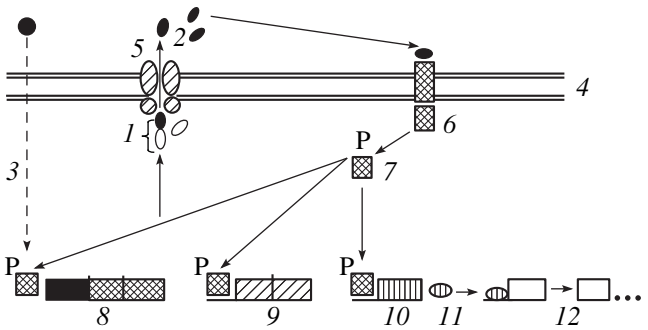

Fig. 3. The synthesis of competence pheromone in streptococci and the regulation of the functions of competence genes (according to [30], modified). *1*, pheromone precursor; *2*, pheromone; *3*, possible effect of the changing medium composition; *4*, cell wall and cytoplasmic membrane; *5*, proteins ComAB; *6*, proteins ComDE; *7*, phosphorylated protein ComE; *8*, *comCDE* operon; *9*, *comAB* operon; *10*, *comX* operon; *11*, *comX* operon product; *12*, late competence genes.

induced by the addition of 10 ng of the pheromone to 6×10^7 cells/ml [29].

The relevant peptides were synthesized proceeding from the nucleotide sequences of their structural genes; the biological activity of the synthetic peptides matched that of the natural peptides. The competence pheromones of various *Streptococcus* species differ in their size, amino acid composition, and species specificity. Moreover, different clinical isolates of the same species may produce differing pheromones (see below). The products of the *comC* gene forming part of the *comCDE* operon are pheromone precursors. The genes of these operons and those of the *comAB* operon belong to the early competence genes. Following is the description of their interaction and the effects on the late competence genes (Fig. 3).

The *comC* gene is responsible for the synthesis of the pheromone precursor that contains 41 amino acid residues in *S. pneumoniae*. It interacts with the peptide export system (the ABC system), which is formed by the products of the *comAB* genes. The pheromone precursor is cleaved at the leader sequence site. The mature pheromone, which is reduced in size by 2.4 times, is liberated from the cell. Thereupon, the two-component pheromone-transferring system comes into play: the pheromone contacts its cell surface receptor (the transmembrane moiety of histidine kinase, the product of the *comD* gene). This is the sensor protein. Apparently, it was the transmembrane moiety of histidine kinase that was described as the inhibitor of the competence factor in the earlier works (see above). The activated histidine kinase phosphorylates the product of the *comE* gene, the response regulator protein, and this phosphorylated protein accumulates in the cell. These processes are carried out at a relatively low baseline rate, and they do not result in competence acquisition.

However, this pattern occurs only at the stage of culture growth characterized by a low cell number and, accordingly, an insignificant pheromone concentration in the medium. As the cells accumulate in the medium, the pheromone amount increases and finally reaches the critical level (slightly above 10 ng/ml). Accordingly, the intracellular amount of phosphorylated protein ComE increases. Once its concentration reaches the threshold level, it binds to the promoter of the *comCDE* operon and stimulates its operation (positive regulation). Autoinduction occurs, and a feedback loop forms: the more pheromone is released into the medium, the more phosphorylated protein ComE forms. It binds to the promoter of its own operon and stimulates its operation, resulting in the formation of additional pheromone amounts, etc. The intensity of the operation of competence operons (primarily of the *comCDE* operon) was quantitatively determined from β-galactosidase synthesis using a reporter gene under the control of the relevant promoter [32–35].

Phosphorylated protein ComE, apart from stimulating its own promoter, also activates the promoter of the *comAB* operon (the secretory system releasing the pheromone from the cell). In addition, it activates the *comX* operon that turns on a chain of late competence genes [30]. As mentioned above, they are responsible for the binding and uptake of transforming DNA and for all the late transformation stages. In *Streptococcus*, these late genes constitute a complex epistatic system termed the competence regulon [33]. This enables the synthesis of a multitude of new proteins mandatory for the completion of all transformation stages. However, the subsequent regulation of cascade synthesis depends on the progressive increase in the concentration of phosphorylated protein ComE. Excessive amounts of this protein at this stage result in the inhibition of the *comCDE* operon against the background of continued stimulation of the operation of the late genes [36]. This causes the deceleration of the synthesis of the protein products of the early competence genes, whereas the products of the late genes are actively synthesized. Due to these concentration effects characteristic of protein ComE, the maximum DNA uptake by the cells occurs with a 5–6 min lag after the release of the pheromone into the medium [35]. Evidently, the protein plays the crucial role in the regulation of transformation by coordinating the operation of the early and the late competence genes.

An increase in the pheromone amount in the culture fluid to the threshold value represents, therefore, the signal resulting in the onset of the complex processes described above. The pheromone increase in turn reflects the growth of the cell number in the population. This enables quorum sensing (see the beginning of this article). Culture cells use the pheromone language to communicate the message “We are sufficiently numerous to make the transition to a new state” (to the competence state). A possible reason why this transition is useful is that the culture accumulates a large amount of DNA that has been released from the cells during autolysis. DNA uptake is the final goal of the transformation

process [36]. Possibly, the baseline level of pheromone synthesis changes in response to the alterations in the medium composition resulting from culture growth [30, 37].

Streptococci produce different kinds of pheromones (see above), which differ in their size and amino acid composition. Two types of pheromones were detected in 42 clinical isolates of *S. pneumoniae*; they were referred to as pherotypes [38]. Nine *Streptococcus* isolates among 11 tested representatives of the mitis 9 group had different pherotypes [39]. Some natural isolates (mutants?) do not synthesize active pheromones. One of the works by R. Pakula (done in the 1960s) used such an isolate (the Wicky strain). This strain became competent under the influence of the pheromone of the Challis strain [15]. Both strains belonged to the *S. gordonii* species. The difference between the pheromones of two different pherotypes may amount to one half of the amino acids they contain.

There are analogous differences between the receptors (histidinase allelomorphs). Each pherotype is characterized by key-lock interactions between the pheromone and the respective histidine kinase (involving the first three coils of its transmembrane moiety). Pheromones can efficiently induce competence only in their own pherotype [31]. Streptococci of various pherotypes resemble eukaryotes that tend to cross within their own population only (sexual isolation). Competence cross-induction between strains with different pherotypes, if at all possible, is three orders of magnitudes less efficient than competence induction inside one pherotype. However, the degree of the net homology of chromosome DNA in strains with different pherotypes can be very high, providing for highly efficient homologous recombination; obviously, the strains belong to the same species. Hence, the pherotype-based classification does not in all cases conform to the traditional classification of streptococci. There may be strains with different pherotypes within one species, and, conversely, different species belonging to the same pherotype. This peculiarity is supposedly due to intense horizontal gene transfer with subsequent recombination in streptococci, resulting in the formation of mosaic genes that contain whole blocks of the other partner's nucleotides [39]. It is unclear why this kind of mosaicism and, accordingly, pherotype hypervariability, mainly occur in the pheromone gene and the part of the histidine kinase gene that is responsible for the transmembrane domain interacting with the pheromone.

COMPETENCE PHEROMONES IN BACILLI

Recent studies on *Bacillus* transformation mainly focused on the *Bac. subtilis* species (the *Bac. subtilis* 168 strain) and, to a considerably lesser extent, on related strains (subspecies) of soil bacilli.

Research on competence pheromones in *Bac. subtilis* dates back to the 1960s. M. Charpac and R. Dedonder [40] in 1965 and A.N. Pariiskaya and E.S. Pukhova [41] two years later independently reported on a competence factor contained in the culture fluid of a competent culture; the factor was partially purified using an ion exchange column. This substance accelerated competence development in *Bacillus* cells at the initial stages of culture growth [42–46]. Like the *Streptococcus* factor, the *Bacillus* competence factor was sensitive to trypsin. The addition of chloramphenicol to a growing culture suppressed the formation of competent cells and of the competence factor per se. However, the data obtained were contradictory in some respects. Some works of the late 1960s presented data that the competence factor exhibited nuclease and lytic activities [47], apparently due to the incomplete purification of the factor. Ambiguous results were also obtained with respect to the thermostability of this substance. The role of the competence factor in *Bacillus* was called into question by some researchers [48].

It was relatively recently that the research on competence pheromones in bacilli received a new impetus, thanks to the studies conducted by A. Grossman *et al.* It was demonstrated that the growth medium of a competent culture contains a small peptide (9–10 amino acid residues). It converts immature cells to competent cells. If the *lacZ* gene is put under the control of the promoter of *srfA*, one of competence operons, then the addition of this peptide induces β-galactosidase synthesis (i.e., the peptide stimulates the operation of competence genes). Determining the changes in the mode of operation of competence genes by monitoring β-galactosidase synthesis was more convenient than directly counting the number of transformant colonies. The peptide was designated ComX [49]. The same research team published a paper one year later describing one more competence-stimulating peptide of a very small size (five amino acids) [50]. This peptide, termed CSF (competence stimulating factor), operated independently of but in combination with peptide ComX.

The mechanism of the influence of both pheromones on the transformation capacity of bacterial cells fits the following scheme (Fig. 4).

The precursor of pheromone ComX is encoded by the small *comX* gene (which should not be confused with the *comX* operon of streptococci: the same designations refer to different genes in streptococci and bacilli). The precursor size is 55 amino acids. While inside the cell, it is modified by another gene, *comQ*, that partially overlaps with *comX*, and released into the intercellular space in the form of an active peptide, a pheromone, which is six times shorter than the precursor. Possibly, its release from the cell is accomplished by a specific transfer system (distinct from that of the *Streptococcus* pheromone). The pheromone interacts thereupon with the transmembrane receptor, a histidine kinase (the product of the *comP* gene in bacilli), resulting in its activation. In response, the histidine kinase

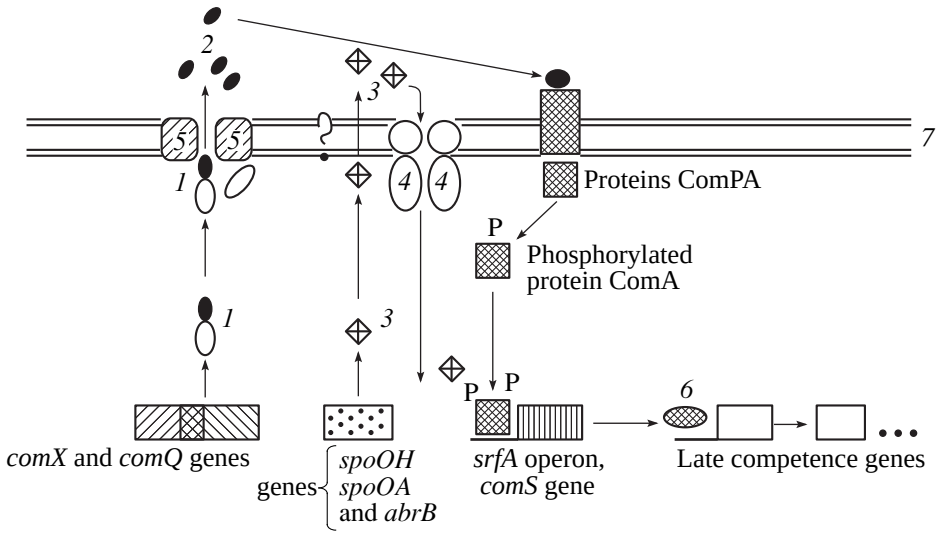

Fig. 4. The synthesis of the competence pheromone in bacilli and the regulation of the functions of competence genes (the figure is based on the relevant hypotheses). *1*, precursor of pheromone ComX; *2*, pheromone ComX; *3*, pheromone CSF; *4*, the product of the *spoOK* gene, an oligopeptide permease; *5*, proteins of the transport system; *6*, the product of the *comS* gene; *7*, cell wall and cytoplasmic membrane.

phosphorylates the relevant regulatory protein ComA, the product of the *comA* gene (a functional analog of protein ComE in streptococci). Upon phosphorylation, this protein binds to the promoter of the *srfA* operon. This results in turning on the complex system of *Bac. subtilis* competence genes (including the late genes responsible for the binding, uptake, and processing of transforming DNA [8, 11]).

The synthesis of the second pheromone, CSF, is apparently subject to regulation by an early sporulation gene (*spoOH*, the gene encoding one of the sigma factors). However, its production is influenced at the transcription level by other genes related to the early sporulation stages, *spoOA* and *abrB*. The small peptide containing five amino acid residues is released from the cell by an unknown mechanism; it reenters the cell with the help of the product of the *spoOK* gene, an oligopeptide permease. Using an unidentified mechanism, it maintains the *comS* gene (whose promoter interacts with phosphorylated protein ComA) in the working condition. Possibly, CSF blocks the phosphatase, preventing ComA dephosphorylation and thereby stimulating the interaction between ComA and the *comS* gene. Hence, the efforts made by both pheromones (ComX and CSF) pursue the same goal of activating the *comS* gene in the *srfA* operon. Since the mutations of some of the genes involved in early sporulation stages also affect CSF production, it is possible that CSF, among other functions, does the sideline job of a signal peptide at early sporulation stages [51].

Hence, there are common features in the behaviors of pheromone ComX in bacilli and the competence pheromone of streptococci. First, both small peptides are extruded into the medium. They induce competence development by activating the whole hierarchy of competence genes in almost all cells of the population (in streptococci) or in a part of the cells (in bacilli). Second, the transfer of such a signal from the extracellular space involves a two-component system: the sensor protein (a histidine kinase) and the response regulator phosphorylated by it. Both pheromones appear in the medium once the culture reaches the threshold cell density; i.e., they are responsible for quorum sensing. However, there are also a number of differences between the two pheromones. Streptococci have no analog of the second *Bacillus* pheromone, CSF. This pheromone extrudes from the cell and thereupon reenters it. It does not give an order via the receptor. Nevertheless, CSF is essential for the development of competence in bacilli. A deletion in the *spoOH* gene decreases 16-fold the transformation capacity of the mutant that fails to produce CSF [52]. In addition, bacilli lack the autocatalytic mechanism enabling the pheromone to stimulate its own synthesis via the sensor protein–response regulator system. Research on competence in bacilli presents difficulties, owing to their sporulation (controlled by over a hundred genes). The gene systems responsible for competence and sporulation partially overlap at early stages and separate stages of both processes are difficult to discern [8, 9, 51]. Therefore, we cannot exclude that the scheme describing the initiation of the competence state in bacilli by pheromones will lose much of its coherence in light of new facts accumulated in the future.

COMPETENCE WITHOUT PHEROMONES AND BACTERIA WITHOUT COMPETENCE

Although natural transformation has been described in more than 50 bacterial species until now, competence pheromones have been detected only in streptococci

and bacilli. Possibly, they have not yet been revealed in some of the species; however, it has been firmly established that transformation in gonococci, meningococci, and hemophilic bacteria, which is sufficiently well understood, proceeds without pheromones. As for gonococci and meningococci, this may be to some extent due to the capacity of their cells to carry out transformation at all growth stages. The transformation capacity is their constant property. In contrast, hemophilic bacteria acquire competence at the early exponential growth phase, i.e., competence is a transient property of this system. However, the competence state is achieved without the prior production of signals related to quorum sensing (such as pheromones) in the cells of these bacteria. It was suggested that the formation of competence pheromones is to some extent due to the desirability of the uptake of conspecific DNA during spontaneous transformation in mixed populations. It was assumed that, at the maximum competence moment, the medium contains the maximum concentration of DNA that is released from cells undergoing autolysis [5]. Hence, a high concentration of free DNA and a functional pheromone concentration may be due to the achievement of a threshold cell number by a bacterial culture. Since streptococci and bacilli do not discriminate between conspecific and alien DNA in a mixed population, this mechanism can signal that there is enough conspecific DNA to engage in taking it up. Hemophilic bacteria can only take up their own DNA, because it includes specific, fairly frequent nine-nucleotide repeats [53]. Therefore, they do not require additional signals concerning the availability of conspecific DNA that is detectable as it is.

As for pheromone-producing bacteria, pheromones form a necessary part of the whole intricate mechanism of competence development. The Wicky isolate of *S. gordonii* (see above), devoid of its pheromone (probably due to a single mutation or a series of them), became competent after the addition of the pheromone of the Challis strain to its culture. Transformation in *S. pyogenes* isolates has not been yet achieved in laboratory conditions. The probable reason is that this group of streptococci lacks the *comC* gene that is responsible for the synthesis of the pheromone precursor in *S. gordonii*. The rest of the competence genes, including the *comDE* genes that control the synthesis of the histidine kinase and the response regulator protein, are operative in this system [27]. Other researchers [39] described clinical isolates belonging to other *Streptococcus* species, which were incapable of acquiring competence because of the disruption of their pheromone system.

Hence, the existence of competence-devoid *Streptococcus* isolates is a sufficiently widespread phenomenon. As for bacilli, we detected only 12 transformation-capable strains among the 118 strains isolated from the soil, which were either similar or identical to *Bac. subtilis* 168 [54]. However, we did not try to establish the reason why 106 strains lacked competence. The question to raise in this context is as follows: How important is the transformation capacity for bacteria? The significant number of genes involved in this process and the perfectly coordinate series of events occurring in the cell during the development of competence suggest that transformation is essential for microorganisms. Based on the data increasingly accumulated in genomics and genetic archaeology, horizontal gene transfer is of paramount importance for the evolution of the genome of many bacteria [55]. However, I do not know of a single work that experimentally demonstrates the selective value of transformation for bacteria under quasinatural conditions. Apparently, an enormous number of generations living in a changing environment is a prerequisite for bacteria to gain the full the advantages of the analogs of sexuality (such as transformation) they possess. Besides, a number of microorganisms probably exist without these advantages.

ACKNOWLEDGMENTS

I wish to thank L. Havarstein and S. Lacks for making reprints of their works available.

REFERENCES

1. Wirth, R., Muscholl, A., and Wanner, G., The Role of Pheromones in Bacterial Interactions, *Trends Microbiol.*, 1996, vol. 4, no. 3, pp. 96–103.
2. Fuqua, W.C., Winans, S., and Greenberg, E., Quorum Sensing in Bacteria: The LuxR–LuxI Family of Cell Density–Responsive Transcriptional Regulators, *J. Bacteriol.*, 1994, vol. 176, no. 2, pp. 269–275.
3. Parkinson, J.S., Kofoid, E.C., Communication Modules in Bacterial Signalling Proteins, *Annu. Rev. Genet.*, 1992, vol. 26. pp. 71–112.
4. Stock, J.B., Ninfa, A.J., and Stock, A.N., Protein Phosphorylation and Regulation of Adaptive Responses in Bacteria, *Microbiol. Rev.*, 1989, vol. 53, no. 4, pp. 450–490.
5. Kaiser, D., Losick, R., How and Why Bacteria Talk to Each Other, *Cell*, 1993, vol. 73, no. 2, pp. 873–885.
6. Salmond, G., Bycroft, B., Stewart, G., and Williams, P., The Bacterial "Enigma": Cracking the Code of Cell–Cell Communication, *Mol. Microbiol.*, 1995, vol. 16, no. 4, pp. 615–624.
7. Kleerebezem, M., Quadri, L., Kuipers, O.P., and de Vos, W., Quorum Sensing by Peptide Pheromones and Two-Component Signal-Transduction Systems in Gram-Positive Bacteria, *Mol. Microbiol.*, 1997, vol. 24, no. 5, pp. 895–904.
8. Dubnau, D., Genetic Competence in *Bacillus subtilis*, *Microbiol. Rev.*, 1991, vol. 55, no. 3, pp. 395–424.
9. Grossman, D.A., Genetic Networks Controlling the Initiation of Sporulation and the Development of Genetic Competence in *Bacillus subtilis*, *Annu. Rev. Genet.*, 1995, vol. 29, pp. 477–508.
10. Prozorov, A.A., Cell Differentiation Related to Genetic Transformation in Bacteria and Its Regulation, *Mikrobiologiya*, 1997, vol. 66, no. 1, pp. 5–13.

11. Prozorov, A.A., DNA Uptake by the Bacterial Cell: The Natural Process and the Laboratory Methods, *Genetika*, 1998, vol. 34, no. 5, pp. 581–592.
12. Pakula, R., Piechovska, M., Bankowska, E., and Walczak, W., A Characteristic of DNA Mediated Transformation of Two Streptococcal Strains, *Acta Microbiol. Polonica*, 1962, vol. 11, no. 3, pp. 205–221.
13. Pakula, R., Cybulska, J., and Walczak, W., The Effect of Environmental Factors on Transformability of a Streptococcus, *Acta Microbiol. Polonica*, 1963, vol. 12, no. 4, pp. 245–258.
14. Pakula, R. and Walczak, W., On the Nature of Competence of Transformable Streptococci, *J. Gen. Microbiol.*, 1963, vol. 31, no. 1, pp. 125–133.
15. Pakula, R., Production of Competence-Provoking Factor and Development of Competence of a Transformable *Streptococcus* in Serum-Free Media, *Can. J. Microbiol.*, 1965, vol. 11, no. 5, pp. 811–822.
16. Leonard, G., Corley, D., and Cole, R., Transformation of Streptococci in Chemically Defined Media, *Biochem. Biophys. Res. Com.*, 1967, vol. 26, no. 2, pp. 181–186.
17. Dobrzanski, W. and Osowiecku, H., Isolation and Some Properties of the Competence Factor from Group H *Streptococcus* Strain Challis, *J. Gen. Microbiol.*, 1967, vol. 48, no. 2, pp. 299–304.
18. Horne, D. and Perry, D., Effect of Competence Induction on Macromolecular Synthesis in Group H *Streptococcus*, *J. Bacteriol.*, 1974, vol. 118, no. 3, pp. 830–836.
19. Tomasz, A. and Hotchkiss, R., Regulation of the Transformability of Pneumococcal Cultures by Macromolecular Cell Products, *Proc. Natl. Acad. Sci. USA*, 1964, vol. 51, no. 3, pp. 480–486.
20. Tomasz, A., Control of the Competent State in *Pneumococcus* by a Hormone-Like Cell Product: An Example for a New Type of Regulatory Mechanism in Bacteria, *Nature*, 1965, vol. 208, no. 5006, pp. 155–159.
21. Cellular Metabolism in Genetic Transformation of Pneumoccocci: Requirement for Protein Synthesis during Induction of Competence, *J. Bacteriol.*, 1970, vol. 101, no. 3, pp. 860–871.
22. Tomasz, A. and Mosser, J., On the Nature of the Pneumococcal Activator Substance, *Proc. Natl. Acad. Sci. USA*, 1966, vol. 55, no. 1, pp. 58–66.
23. Morrison, D. and Baker, M., Competence for Genetic Transformation in *Pneumococcus* Depends on the Synthesis of a Small Set of Proteins, *Nature*, 1979, vol. 282, no. 5735, pp. 215–217.
24. Raina, J. and Ravin, A., Switches in Macromolecular Synthesis during Induction of Competence for Transformation of *Streptococcus sanguis*, *Proc. Natl. Acad. Sci. USA*, 1980, vol. 77, no. 10, pp. 6062–6066.
25. Morrison, D.A., Competence-Specific Protein Synthesis in *Streptococcus pneumoniae*, *Transformation-1980*, Polsinelli, M. and Mazza, G., Eds., Oxford: Cotswold, 1980, pp. 39–54.
26. Vijayakumar, M.N. and Morrison, D.A., Localization of Competence-Induced Proteins in *Streptococcus pneumoniae*, *J. Bacteriol.*, 1986, vol. 165, no. 2, pp. 689–695.
27. Havarstein, L.S. and Morrison, D.A., Quorum-Sensing and Peptide Pheromones in Streptococcal Competence for Genetic Transformation, Dunny, C.M. and Winans, S.C., Eds., Washington: Am. Soc. Microbiol., 1999, pp. 9–26.
28. Havarstein, L.S., Coomaraswamy, G., and Morrison, D.A., An Unmodified Heptadecapeptide Pheromone Induces Competence for Genetic Transformation in *Streptococcus pneumoniae*, *Proc. Natl. Acad. Sci. USA*, 1995, vol. 92, no. 24, pp. 11140–11144.
29. Chen, J. and Morrison, D.A., Modulation of Competence for Genetic Transformation in *Streptococcus pneumoniae*, *J. Gen. Microbiol.*, 1987, vol. 133, no. 5, pp. 1959–1967.
30. Lee, M. and Morrison, D.A., Identification of a New Regulator in *Streptococcus pneumoniae* Linking Quorum Sensing to Competence for Genetic Transformation, *J. Bacteriol.*, 1999, vol. 181, no. 16, pp. 5004–5016.
31. Havarstein, L.S., Gaustad, P., Nes, I., and Morrison, D.A., Identification of the Streptococcal Competence Pheromone Receptor, *Mol. Microbiol.*, 1996, vol. 21, no. 4, pp. 863–869.
32. Pestova, E.V., Havarstein, L.S., and Morrison, D.A., Regulation of Competence for Genetic Transformation in *Streptococcus pneumoniae* by an Auto-Induced Peptide Pheromones and a Two-Component Regulatory System, *Mol. Microbiol.*, 1996, vol. 21, no. 4, pp. 853–862.
33. Campbell, E., Choi, S., and Masure, H.R., A Competence Regulon in *Streptococcus pneumoniae* Revealed by Genomic Analysis, *Mol. Microbiol.*, 1998, vol. 27, no. 5, pp. 929–939.
34. Alloing, G., Granadel, C., Morrison, D.A., and Claverys, J.P., Competence Pheromone, Oligopeptide Permease, and Induction of Competence in *Stretococcus pneumoniae*, *Mol. Microbiol.*, 1996, vol. 21, no. 3, pp. 471–478.
35. Alloing, G., Martin, B., Granadel, G., and Claveris, J.P., Development of Competence in *Streptococcus pneumoniae*: Pheromone Autoinduction and Control of Quorum-Sensing by the Oligopeptide Permease, *Mol. Microbiol.*, 1998, vol. 9, no. 1, pp. 75–83.
36. Ween, O., Gaustad, P., and Havarstein, L.S., Identification of DNA Binding Sites for ComE, a Key Regulator of Natural Competence in *Streptococcus pneumoniae*, *Mol. Microbiol.*, 1999, vol. 33, no. 4, pp. 817–827.
37. Pearce, B.J., Naughton, A., and Masure, H., Peptide Permeases Modulate Transformation in *Streptococcus pneumoniae*, *Mol. Microbiol.*, 1994, vol. 21, no. 6, pp. 881–892.
38. Pozzi, G., Masala, L., Jannelli, F., Manganelli, R., Havarstein, L.S., Piccoli, L., Simon, D., and Morrison, D.A., Competence for Genetic Transformation in Encapsulated Strains of *Streptococcus pneumoniae*: Two Allelic Variants of the Peptide Pheromone, *J. Bacteriol.*, 1996, vol. 178, no. 20, pp. 6087–6090.
39. Havarstein, L.S., Hakenbeck, R., and Gaustad, P., Natural Competence in the Genus Streptococcus: Evidence That Streptococci Can Change Pherotype by Interspecies Recombinational Exchange, *J. Bacteriol.*, 1997, vol. 179, no. 21, pp. 6589–6594.
40. Charpac, M. and Dedonder, R., Production d'un "facteur competence" soluble par *Bacillus subtilis* Marburg 168, *Compt. Rend. Acad. Sci.*, 1965, vol. 260, no. 21, pp. 5638–5641.

41. Pariiskaya, A.A. and Pukhova, E.S., On the Competence Factor of *Bacillus subtilis*, *Mikrobiologiya*, 1967, vol. 36, no. 3, pp. 456–463.

42. Akrigg, A., Ayad, S., and Barker, G., The Nature of a Competence-Inducing Factor in *Bacillus subtilis*, *Biochem. Biophys. Res. Com.*, 1967, vol. 28, no. 6, pp. 1062–1067.

43. Arkigg, A. and Ayad, S., The Competence-Inducing Factor of *Bacillus subtilis*, *Biochem. J.*, 1969, vol. 112, no. 1, pp. 13–16.

44. Ayad, S. and Scimmin, E., The Existence of Two Forms of the Competence Factor in *Bacillus subtilis*, *Biochem. Soc. Trans.*, 1973, vol. 1, no. 3, pp. 705–707.

45. Ayad, S. and Shimmin, E., The Lytic Properties of the Two Forms of the Competence-Inducing Factor in *Bacillus subtilis*, *Biochem. Soc. Trans.*, 1973, vol. 1, no. 4, pp. 864–866.

46. Joenje, H., Graber, M., and Venema, G., Stimulation of the Development of Competence by Culture Fluids in *Bacillus subtilis* Transformation, *Biochim. Biophys. Acta*, 1972, vol. 262, no. 2, pp. 189–199.

47. Finn, Ch. and Landman, O., Competence Related Proteins in the Supernatant of Competence Cells of *Bacillus subtilis*, *Mol. Gen. Genet.*, 1985, vol. 198, no. 2, pp. 329–335.

48. Goldsmith, M., Havas, S., Ma, R.-J., and Kallenbach, N., Intercellular Effect on Development of Competence in *Bacillus subtilis*, *J. Bacteriol.*, 1970, vol. 102, no. 3, pp. 774–783.

49. Magnuson, R., Solomon, J., and Grossman, A.D., Biochemical and Genetic Characterization of a Competence Pheromone from *Bacillus subtilis*, *Cell*, 1994, vol. 77, no. 2, pp. 207–216.

50. Solomon, J., Magnuson, R., Srivastava, A., and Grossman, A.D., Convergent Sensing Pathways Mediate Response to Two Extracellular Competence Factors in *Bacillus subtilis*, *Gene Devel.*, 1995, vol. 9, no. 5, pp. 547–558.

51. Solomon, J. and Grossman, A.D., Who's Competent and When: Regulation of Natural Genetic Competence in Bacteria, *Trends Genet.*, 1996, vol. 12, no. 1, pp. 150–155.

52. Albano, M., Hahn, J., and Dubnau, D., Expression of Competence Genes in *Bacillus subtilis*, *J. Bacteriol.*, vol. 169, no. 11, pp. 3110–3117.

53. Prozorov, A.A., The Bacterial Genome: Nucleoid, Chromosome, and Nucleotide Map, *Mikrobiologiya*, 1998, vol. 67, no. 4, pp. 437–451.

54. Chan Kam Van, Kuzin, Yu.Yu., Kozlovskii, Yu.E., and Prozorov, A.A., A Study of the Capacity for Genetic Transformation in Soil Isolates Close to *Bacillus subtilis* 168, *Genetika*, 1985, vol. 21, no. 12, pp. 1953–1959.

55. Prozorov, A.A., Horizontal Gene Transfer in Bacteria: Laboratory Modeling, Natural Populations, and Data from Genome Analysis, *Mikrobiologiya*, 1999, vol. 68, no. 5, pp. 632–646.

56. Lacks, S.A., DNA Uptake by Transformable Bacteria, *Transport of Molecules across Microbial Membranes*, Broome-Smith, J. *et al.*, Eds., Cambridge Univ. Press, 1999, pp. 139–188.