Properties and Biological Role of Streptococcal Fratricins

Kari Helene Berg, Truls Johan Biørnstad, Ola Johnsborg,* and Leiv Sigve Håvarstein

Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, Ås, Norway

Competence for natural genetic transformation is widespread in the genus Streptococcus. The current view is that all streptococcal species possess this property. In addition to the proteins required for DNA uptake and recombination, competent streptococci secrete mureinolytic enzymes termed fratricins. Since the synthesis and secretion of these cell wall-degrading enzymes are always coupled to competence development in streptococci, fratricins are believed to carry out an important function associated with natural transformation. This minireview summarizes what is known about the properties of fratricins and discusses their possible biological roles in streptococcal transformation.

Streptococcus pneumoniae and several related species in the Mitis phylogenetic group, i.e., Streptococcus mitis, Streptococcus oralis, Streptococcus sanguinis, Streptococcus cristatus, and Streptococcus infantis, have been known to be competent for natural genetic transformation for decades. Other species in the genus Streptococcus that have this property include Streptococcus mutans and all of the members of the Anginosus phylogenetic group (41). Competent streptococci do not discriminate between homologous and foreign DNAs. They take up any extracellular DNA (eDNA), regardless of its source. Competence develops spontaneously during the early logarithmic phase in laboratory-grown S. pneumoniae cultures and lasts 40 to 60 min. In other streptococcal species, however, the period of competence might last several hours (45, 77).

For the majority of streptococcal species, competence for natural transformation has never been demonstrated in the laboratory. Nevertheless, all streptococci appear to possess the core competence genes encoding the proteins required for the uptake and integration of exogenous DNA. Transcription of the core competence genes, which belong to the late competence genes, is controlled by the alternative sigma factor ComX (47). Similar to the core competence genes, ComX is conserved in streptococci, strongly indicating that competence is more widespread in this genus than established by experiments. Some recent developments support this view. In 2006, Blomqvist and coworkers showed that artificial overexpression of ComX in Streptococcus thermophilus induced the competent state in this dairy species, demonstrating that its core competence genes are intact and functional (8). S. thermophilus differs from naturally transformable species of the Mitis and Anginosus groups in that it lacks the ComABCDE competence induction pathway. This quorum-sensing-like pathway consists of the competence-stimulating peptide (CSP) encoded by comC, its secretion and processing apparatus comAB, its transmembrane receptor comD, and the cognate response regulator ComE (35, 36, 40, 57, 76). The absence of the ComABCDE signaling pathway from S. thermophilus suggested that expression of the competence master switch, ComX, is controlled by a different mechanism in this species. This turned out to be exactly the case. The features of the new competence induction pathway, which is in the following termed ComRS, were reported in two successive papers (27, 28). These findings spurred the interest of other researchers, who were able to show that S. mutans contains a ComRS pathway in addition to the previously identified pathway of the ComABCDE type (49). Both pathways are able to induce the competent state in S. mutans, but ComR, rather than ComE, is the proximal regulator of ComX. Mashburn-Warren et al. (49) also provided evidence that competence in a number of streptococcal species belonging to the pyogenic and Bovis phylogenetic groups, is regulated by ComRS-like pathways. Thus, recent progress in this field clearly indicates that most, or probably all, species in the genus Streptococcus are competent for natural genetic transformation.

All of the naturally transformable streptococci investigated so far possess (i) a set of core competence genes controlled by the competence-specific sigma factor ComX, (ii) a pheromone-sensing signal transduction pathway that induces the expression of ComX, and (iii) accessory regulatory mechanisms that respond to various internal and external cues and modify the level or activity of ComX (Fig. 1). The latter are, in general, poorly understood, but in S. pneumoniae, these accessory regulatory mechanisms include ComW, HtrA, Clp proteins, the CiaRH two-component system, and the Ser/Thr protein kinase StkP (12, 20, 30, 32, 48, 67, 72). In addition to the shared characteristics described above, all streptococci appear to express a murein hydrolase during competence (3). As illustrated in Fig. 2, distinct types and subtypes of murein hydrolases are produced by different streptococcal species. These murein hydrolases are part of the ComX regulon, often called the late competence genes, but they are not essential for the uptake and incorporation of transforming DNA. Nevertheless, these competence-specific murein hydrolases are omnipresent in the genus Streptococcus. For this reason, it is reasonable to assume that they carry out a very important function associated with natural transformation in streptococci. Here we give a summary of the recent advances in this area and present our view on the biological function of competence-associated murein hydrolases.

COMPETENCE-INDUCED FRATRICIDE IN S. PNEUMONIAE

Ten years ago, Steinmoen et al. (70) reported that induction of the competence state in a culture of S. pneumoniae cells results in lysis and release of DNA from a subfraction of the population. They also showed that DNA release and uptake have essentially the
transcription of these genes. Pre-ComS, the product of the comS gene, is secreted by an unidentified transporter and is presumably processed into the mature pheromone ComS*. After it has been translocated across the cytoplasmic membrane, the exact processing site of pre-ComS is not known, but the eight C-terminal amino acids of the precursor have biological activity. Extracellular ComS*, which serves as a quorum-sensing signal, is imported into the cells by the oligopeptide Ami transporter. Inside the cell, it binds to and activates the transcriptional regulator ComR. Activated ComR binds to an inverted repeat motif (ECom box) in the promoter regions of the comR and comS genes, resulting in amplification of the ComS* signal and expression of the late competence genes (27). ClpCP and MecA act together or separately to prevent accumulation of ComX under conditions that are suboptimal for competence genes (27). ClpC and MecA act together or separately to prevent cell lysis is abolished in the absence of this protein. In the absence of LytA or LytC, the process becomes less efficient but neither LytA nor LytC is an essential component of the mechanism. A likely scenario, which is supported by experimental evidence, is that CbpD introduces specific cuts into the peptide stems of pneumococcal peptidoglycan that allow LytC and LytA to become active (21, 31, 56).

CbpD is a modular murein hydrolase that consists of an N-terminal CHAP domain, two central Src homology 3b (SH3b) domains, and a C-terminal choline-binding domain (CBD) made up of four choline-binding repeats (Fig. 2) (22). The choline-binding repeats bind noncovalently to teichoic acid present in the cell envelope of S. pneumoniae. The teichoic acid type produced by S. pneumoniae is complex and very unusual. The repeating unit consists of a tetrasaccharide glycosydically linked to C-1 of ribitol (2). The wall teichoic acids (WTAs) and lipoteichoic acids (LTAs), often called C-polysaccharide and F-polysaccharide, respectively, have identical structures, except for the part that anchors LTA to the membrane (24). This is not the case in most other Gram-positive bacteria, where the WTAs and LTAs are structurally different. Another unusual feature of pneumococcal teichoic acids is that both WTAs and LTAs are decorated with phosphorylcholine residues. These residues serve as attachment sites for surface proteins containing CBDs (65). Hence, the C-terminal CBD of CbpD enables it to attach to the teichoic acid portion of the pneumococcal cell envelope. Recent evidence indicates that the SH3b domains direct the catalytic CHAP domain to its peptidoglycan substrate by binding to an as-yet-unknown part of this macromolecule (22). Mutational analyses have shown that the SH3b domains and the CBD domain are essential for the functionality of CbpD (22). The CHAP domain of CbpD belongs to a very large same kinetics, suggesting that lysed cells might act as donors of transforming DNA to the surviving competent cells in the population. Based on these results, the authors speculated that the competence-induced lysis mechanism (later termed fratricide) serves to facilitate exchange of DNA between pneumococci in nature. The observation that addition of 2% choline to the competent culture abolished cell lysis strongly suggested that a choline-binding protein is involved. Further research showed that three choline-binding murein hydrolases, CbpD, LytA, and LytC, constitute the lysis mechanism in S. pneumoniae. CbpD was identified as the key component of the mechanism, while LytA and LytC play auxiliary roles (21, 31, 43). The genes encoding CbpD, LytA, and LytC are located in three separate transcription units. The central role of CbpD is illustrated by the fact that competence-induced cell lysis is abolished in the absence of this protein. In the absence of LytA or LytC, the process becomes less efficient but neither LytA nor LytC is an essential component of the mechanism. A likely scenario, which is supported by experimental evidence, is that CbpD introduces specific cuts into the peptide stems of pneumococcal peptidoglycan that allow LytC and LytA to become active (21, 31, 56).
family of cysteine, histidine-dependent amidohydrolases/peptidases, most of which are involved in cell wall hydrolysis. The exact bond split by CbpD has not been identified, but in those cases where the cleavage sites of muralytic CHAP domains have been determined, they act either as endopeptidases that cleave within murein stem peptides or as amidases that cleave the N-acetylmuramyl-L-Ala bond (1, 46, 64).

LytA, the major autolysin of *S. pneumoniae*, is an acetylmutamyl-L-alanine amidase. It contributes to virulence, probably by functioning as a release mechanism for the cytolytic toxin pneumolysin, and is responsible for the stationary-phase lysis of pneumococci. LytC, which is a lysozyme, has no known biological function except for its auxiliary role in CbpD-mediated lysis of susceptible cells (56). In addition to their catalytic domains, LytA and LytC contain CBDs at their C- and N-terminal ends, respectively. The genes encoding CbpD and LytA are both part of the ComX regulon (15, 59). However, whereas CbpD is expressed only during competence, transcription of the *lytA* gene is driven by several promoters, one of which is competence inducible (51). Consequently, transcription of the *lytA* gene increases in competent pneumococci. In contrast, the level of *lytC* transcription remains unaltered during the competent state (15, 59).

To avoid committing suicide, competent pneumococci express an immunity protein, ComM, which protects them against the muralytic activity of CbpD by an unknown mechanism (22, 37). ComM is a membrane-embedded protein with no close homologs in the protein databases. Since the gene encoding ComM belongs to the early competence genes, it is expressed about 5 min before CbpD, presumably to give the cells time to become immune. CbpD has been shown to bind and attack the septal region of target cells (22). It is therefore possible that ComM modifies the structure of the most newly synthesized portion of the cell wall to make it refractory to degradation by CbpD. Zymogram analyses have provided some support for this theory. When protein samples containing CbpD were subjected to SDS-PAGE with heat-inactivated noncompetent or competence-induced cells in the resolving gel, a clearing zone corresponding to the muralytic activity of CbpD appeared much earlier in the gel containing noncompetent cells than in the gel containing competence-induced cells (unpublished results).

Why is competence for natural genetic transformation in *S. pneumoniae* coregulated with a lytic enzyme that kills and lyses noncompetent sister cells? As mentioned above, it has been speculated that this could be a predatory gene acquisition mechanism used by pneumococci to capture DNA from other pneumococcal strains or closely related species (70). Johnsborg and coworkers (42) tested this idea by performing gene transfer experiments with mixed cultures of competent attacker and noncompetent target cells. It turned out that CbpD-proficient attacker cells were 1,000-fold more efficient than CbpD-deficient attacker cells in capturing an antibiotic resistance marker from noncompetent target cells (42). Experiments were also carried out to determine whether pneumococcal CbpD is active against *Streptococcus mitis* and *S. oralis*. The results showed that pneumococcal attackers with a functional *cbpD* gene are 40-fold more efficient at capturing DNA from *S. oralis* SK153 than *cbpD*-deficient attacker cells are. In an identical experiment where *S. mitis* NCTC 12261 was used as the target strain, the difference was 10-fold (42). The CbpD proteins produced by *S. pneumoniae*, *S. oralis*, and *S. mitis*, which are called CbpD-Sp, CbpD-So, and CbpD-Sm here, are highly homologous and carry the same domains. They differ only in the number of SH3b domains they contain. Strains of *S. oralis* and *S. mitis* produce CbpD proteins with one SH3b domain, whereas the number of such domains varies between one and two.

**FIG 2** Domain organization of σ^X^-controlled murein hydrolases from different species of streptococci. CHAP, cysteine, histidine-dependent amidohydrolases/peptidases (1, 64); SH3, binds peptidoglycan (22); choline-binding repeats, binds choline residues linked to teichoic acid (65); Cons. (conserved) domain, uncharacterized domain that probably mediates binding to the cell wall of target cells; RICIN, carbohydrate binding domain; peptidase M_23, zinc metallopeptidases with a range of specificities. Reprinted from reference 3.
in pneumococcal strains (Fig. 2). Searches of databases show that highly similar CbpD proteins with the same domain composition are also encoded in the genomes of *Streptococcus pseudopneumoniae*, *Streptococcus infantis*, and *Streptococcus peroris* (Fig. 2). In order to be active, CbpD proteins with CBDs require that the teichoic acids of target cells contain phosphorylcholine. A survey of a number of streptococcal isolates, identified as *S. pneumoniae*, *S. pseudopneumoniae*, *S. mitis*, or *S. oralis*, showed that virtually all of them reacted with a phosphorylcholine-specific monoclonal antibody designated HAS. The exception was 5 out of 57 isolates belonging to the *S. pneumoniae-S. mitis*-S. pseudopneumoniae cluster. In addition, some of the strains identified as *S. infantis* did not react with the HAS monoclonal antibody (44). Genome sequence data support the idea that members of the above-mentioned species possess choline-decorated teichoic acids (17, 19). Based on these results and the high similarity of the CbpD proteins of *S. pneumoniae*, *S. pseudopneumoniae*, *S. mitis*, *S. oralis*, *S. infantis*, and *S. peroris* (Fig. 2), it is likely that CbpD-mediated cross-species lysis is common within this group of streptococcal species.

**CbpD PROTEINS WITHOUT CBDs**

Searches of protein databases with the pneumococcal CHAP domain as a query sequence revealed that many species in the genus *Streptococcus* encode CbpD-like proteins that lack a CBD (3, 13). All of them have typical ComX binding sites in their promoter regions, strongly indicating that they are expressed during competence. Some of them, such as those encoded by *Streptococcus suis* and several members of the pyogenic phylogenetic group, contain SH3b domains, while those encoded by *Streptococcus thermophilus*, *Streptococcus salivarius*, *Streptococcus vestibularis*, *Streptococcus criceti*, and *Streptococcus downei* do not (Fig. 2). Presumably, these CbpD-like proteins are functional analogues of CbpD-Sp that use other types of domains to recognize their respective target cells. The CbpD-St protein encoded by *S. thermophilus*, for instance, contains a C-terminal region that is totally unrelated to the C-terminal region of CbpD-Sp. Zymogram analyses in which *S. thermophilus* cells were incorporated in the resolving gel as the substrate revealed that CbpD-St is a murein hydrolase that is produced only during competence. Furthermore, examination of the cell surface-binding properties of a fusion protein in which the CHAP domain had been exchanged with green fluorescent protein (GFP) demonstrated that the C-terminal domain of CbpD-St specifically attaches this protein to the equatorial region of *S. thermophilus* cells (7). Evidently, the CbpD-like proteins depicted in Fig. 2 are chimeras that have been assembled from a catalytic domain and one or more cell surface-binding domains with specificities that limit their target range. Zymogram analysis shows that CbpD-St has no activity against *S. pneumoniae* cells (unpublished data). This result indicates that the CHAP domains of CbpD-St and CbpD-Sp have different cleavage specificities or that their C-terminal regions recognize different structures in the envelopes of their target cells. The latter possibility is supported by the findings that CbpD-Sp and CbpD-St display similar but non-identical binding patterns. CbpD-Sp binds to the equator, septum, and poles of pneumococci, while CbpD-St binds predominantly to the equatorial region of *S. thermophilus* cells (7, 22).

**IDENTIFICATION OF A NEW TYPE OF FRATRICIN**

Many streptococcal species possess fratricins of the type discussed above, but database searches revealed that a significant number of species do not encode CbpD-like proteins (3, 13). Does this mean that competence is not coupled with the production of muranolytic enzymes in these species, or do they produce a different murein hydrolase that carries out the same function? Intriguingly, in a recent report, Berg et al. (3) showed that all species lacking a CbpD-like fratricin (except *Streptococcus agalactiae*) possess an alternative competence-induced murein hydrolase termed LytF (Fig. 2). LytF proteins consist of an N-terminal signal peptide, 2 to 5 Bsp-like (group B streptococcal secreted) protein domains, and a C-terminal CHAP domain. The CHAP domains of LytF proteins share little sequence similarity with the CHAP domains of CbpD-like proteins. Using *Streptococcus gordonii* strain Challis as a model organism, Berg et al. (3) showed that LytF binds to the equator, septum, and poles of this strain via its Bsp domains. This binding pattern is strikingly similar to that displayed by CbpD-Sp (22). Cocultivation experiments between *S. gordonii* strains Challis and NCTC 7865, carrying a streptomycin and a rifampin marker, respectively, showed that the presence of LytF has a large positive impact on the number of Str<sup>+</sup> Rif<sup>+</sup> double-resistant transformants obtained. Transfer of the rifampin marker from noncompetent NCTC 7865 cells to competent Challis cells was 100-fold more efficient with LytF-proficient Challis cells than with LytF-deficient Challis cells (3). These results indicate that despite their different origins, CbpD-Sp and LytF have been recruited to serve the same function in their respective species.

**FRATRICINS: WHAT ARE THEY FOR?**

Experimental data and *in silico* analyses strongly indicate that bacteriolytic fratricins are produced by all streptococcal species during competence for natural genetic transformation, implying that these enzymes play an important role in this process. What are streptococci trying to accomplish by producing these murein hydrolases? In our view, there are four possibilities: (i) facilitation of DNA uptake, (ii) chemical warfare to eradicate competing bacteria, (iii) acquisition of donor DNA from lysed target cells, and (iv) release of DNA from lysed target cells to provide structural support for biofilm formation.

**DO FRATRICINS HAVE A ROLE IN DNA UPTAKE?**

In order to reach the cytoplasm, transforming DNA has to cross the cell wall of competent streptococci. Although most or perhaps all of the components of the DNA uptake machinery have been identified, it is not fully understood how DNA is translocated across the peptidoglycan layer. Thus, it is still an open question whether this process requires rearrangements within the peptidoglycan (18). What is certain is that type IV pseudopili or pilus-like structures constitute an essential part of the DNA uptake machinery. It is thought that repeated cycles of polymerization and depolymerization of these pseudopili provide the mechanical force that pulls the DNA strand across the cell wall, perhaps by direct interaction between the pilus and DNA (11, 14). During polymerization, the pili are gradually extended through the peptidoglycan layer. Do they penetrate the cell wall through existing pores, or are larger pores created within the peptidoglycan sacculus by a murein hydrolase dedicated to pilus assembly? Using an experimental approach, Demchick and Koch estimated that the effective pore size in walls from *Bacillus subtilis* is 2.12 nm (16), while Meroueh et al. (50) calculated that the smallest pores in peptidoglycan are 7 nm across. Since the extent of cross-linking in bacterial peptidoglycan varies considerably, it is likely that larger
pores exist as well (75). Type IV pseudopili, which consist of helical assemblies of pilin subunits, are about 6 nm in diameter (55). Judging from these data, type IV pseudopili might be able to penetrate the peptidoglycan layer without the assistance of a muralytic enzyme. On the other hand, as specialized lytic transglycosylases are known to be required for insertion of cell wall-spanning structures such as secretion systems and flagella (66), it cannot be ruled out that efficient assembly of pseudopili in competent pneumococci depends on a cell wall-degrading enzyme. Could the biological role of CbpD-Sp, CbpD-St, and LytF be to create room for plius assembly? If so, deletion of the genes encoding these muraline hydrolases should abolish or at least significantly reduce the transformability of their respective species. Experiments carried out to compare the transformability of a cbpD-Sp deletion mutant and that of its parental strain revealed that there is no significant difference between the two (42). Similarly, no difference in transformation efficiency was detected when an S. gordonii lytF mutant and the corresponding wild-type strain were subjected to genomic DNA carrying an antibiotic resistance marker (3). These results clearly show that DNA uptake in S. pneumoniae and S. gordonii does not depend on CbpD-Sp or LytF and therefore argue strongly against a role for these muraline hydrolases in assembly of the DNA uptake apparatus. In contrast, depending on the experimental setup used, deletion of the cbpD-St gene in S. thermophilus resulted in a 6- to 18-fold reduction in transformation efficiency (7). The presence of CbpD-St is not required for transformation, but it stimulates the process. What could be the explanation for these conflicting results? One possibility is that CbpD-St and CbpD-Sp/LytF have evolved different functions. S. thermophilus, which was domesticated several thousand years ago, is extensively used by the dairy industry to produce yoghurt and cheeses. The dairy niche, which contains very few bacterial species, is completely different from the multispecies biofilms occupied by S. gordonii and S. pneumoniae. Thus, it is conceivable that the original function of CbpD-St became redundant after S. thermophilus was domesticated and that it subsequently acquired a function in DNA uptake. Investigation of the properties of the CbpD proteins from the oral commensals Streptococcus vestibularis and Streptococcus salivarius, the most closely related nondomesticated relatives of S. thermophilus, could help resolve these matters. However, even though CbpD-St has a stimulatory effect on the transformability of S. thermophilus in laboratory experiments, the possibility cannot be excluded that the primary function of CbpD-St is to act as a fratricin. Its positive effect on the transformation rate in laboratory experiments might just be a side effect of its muralytic activity (see further discussion below).

ARE FRATRICINS ACTUALLY CLASS III BACTERIOCINS?

Bacteria produce a plethora of antimicrobial metabolites that are used to kill competing microorganisms sharing the same niche. Are streptococcal fratricins just part of this chemical arsenal, or do they serve other or additional functions? It is well established that streptococci produce a number of different ribosomally synthesized antimicrobial compounds, so-called bacteriocins. Most of these bacteriocins are heat-stable peptides composed of 20 to 60 amino acid residues. They are grouped into two major classes, I and II, consisting of the posttranslationally modified lantibiotics and the linear unmodified nonlantibiotics, respectively (54). In addition, a third class has been proposed that consists of bacteriolytic muraline hydrolases. So far, only three streptococcal class III bacteriocins have been described: zoozin A (ZooA) produced by Streptococcus equi subsp. zooepidemicus, millericin B (MilB) produced by Streptococcus milleri (now classified as Streptococcus anginosus), and stellalysin (StlA) produced by Streptococcus constellatus (5, 23, 38, 68). These muraline bacteriocins are made by only a few strains of their respective species. Similar to the fratricins, the three bacteriocins consist of a catalytic domain and a cell wall-targeting domain. Zoozin A and stellalysin display significant homology (72% identity), and both contain a catalytic domain belonging to the M23 family of metallopeptides (38). Cell wall-degrading peptides in this family cleave either the N-acetylmuramyl-1-4 bond between the cell wall peptidoglycan and the cross-linking peptide or a bond within the cross-linking peptide. The catalytic domain of millericin B belongs to the same family and is almost identical to the corresponding domain of lysostaphin from Staphylococcus simulans. The zooA, stlA, and milB genes are in all cases flanked by immunity genes encoding Fem-ABX-like proteins. Members of this family, which includes the streptococcal MurMN proteins, catalyze the synthesis of interpeptide bridges by adding amino acids to the stem peptides (5, 25, 29, 38). Thus, self-immunity against ZooA, StlA, and MilB is obtained by altering the structure of the stem peptides, i.e., the substrate of these muraline bacteriocins.

Searches in the genome of Streptococcus agalactiae revealed that it contains a zooA gene with a typical ComX binding site in its promoter region (Fig. 2). Since S. agalactiae is the only member of the genus Streptococcus that lacks a competence-induced muraline hydrolase of the CbpD or LytF type, it seems likely that zoozin A functions as a fratricin in this species (3). Interestingly, no potential immunity gene is located upstream or downstream of the S. agalactiae zooA gene. Similarly, no immunity gene has been identified that protects S. thermophilus against CbpD-St or S. gordonii against LytF. So far, a fratricin-specific immunity protein has been identified only for S. pneumoniae, S. pseudopneumoniae, S. mitis, S. oralis, S. infantis, and S. peroris, which all carry the comM gene. The functional unit represented by the muralytic enzyme and the corresponding self-protection mechanism is differently organized in fratricins and class III bacteriocins. The genes encoding class III bacteriocins and their respective immunity genes are always located next to each other, while a fratricin gene is never cotranscribed with or located next to an immunity gene. This suggests different biological roles for the two systems. Furthermore, the fact that fratricins are always associated with competence development in streptococci indicates that they have a specific function associated with natural genetic transformation. If fratricins were purely chemical weapons used for interspecies competition, they would be expected to exhibit a relatively broad inhibition spectrum. This is clearly not the case. The CbpD proteins containing CBDs, for instance, are active only against streptococci containing choline-decorated teichoic acids in their cell walls. This narrow target range makes sense if it is assumed that competent streptococci produce fratricins in order to capture homologous DNA from closely related strains and species. What, then, is the biological function of fratricins, warfare or DNA acquisition? These alternatives are not mutually exclusive. The fratricide mechanism might therefore serve both functions at the same time.
ARE FRATRICINS USED FOR ACQUISITION OF HOMOLOGOUS DNA?

According to the estimates of Bolotin et al. (9), S. thermophilus adapted to the dairy niche several thousand years ago. This niche is characterized by constant, well-defined, growth conditions and a lack of competing species. It was therefore not surprising that whole-genome sequencing of S. thermophilus CNRZ1066 and LMG18311 revealed a striking level of gene decay (~10%) (9). Intriguingly, competence for natural genetic transformation was not lost in S. thermophilus during adaptation to the dairy niche, demonstrating that this DNA uptake mechanism is important for survival also in the milk environment. In yoghurt production, S. thermophilus is used together with Lactobacillus delbrueckii subsp. bulgaricus. The combined use of these two bacteria has led to the development of a symbiotic relationship between them (39), and it is therefore unlikely that CbpD-St is used as a chemical weapon against L. delbrueckii subsp. bulgaricus. A fusion protein consisting of GFP and the C-terminal cell wall-targeting domain of CbpD-St does not bind to S. gordonii and S. sanguinis (unpublished results). Thus, similar to CbpD-Sp and LytF (3, 42), CbpD-St probably has a narrow target range. For this reason, it is likely that CbpD-St is inactive against L. delbrueckii subsp. bulgaricus and other bacterial species used together with S. thermophilus in dairy fermentation.

During their partnership in yoghurt production, it appears that S. thermophilus has acquired the metC gene from L. delbrueckii subsp. bulgaricus. This allows S. thermophilus to synthesize methionine, a rare amino acid in milk (9). Does this mean that acquisition of new genetic information from L. delbrueckii subsp. bulgaricus and other species used in the production of yoghurt or so-called hard “cooked” cheeses is the reason why natural transformation has been conserved in S. thermophilus? Considering that its partners in dairy fermentations are genetically unrelated to S. thermophilus, uptake of foreign DNA from these bacteria will, in general, not be beneficial. DNA from unrelated bacteria could, in principle, be used for nutritional purposes. However, the fact that the products of late competence genes such as sbbB, dprA, and recA protect incoming single-stranded DNA from degradation and prepare it for recombination with the recipient’s genome clearly shows that natural transformation is not a food-gathering mechanism (4, 52). A plausible reason that competence for natural transformation has been conserved in S. thermophilus is that it is important for recombinational DNA repair. Presumably, competent S. thermophilus cells secrete CbpD-St to lyse their sister cells and capture their DNA. Experiments have shown that about 4% of the cells in a liquid culture of S. thermophilus are lysed when the culture is induced to competence by the addition of ComS'. CbpD-St is very sensitive to oxidative inactivation by H₂O₂ and it is therefore likely that the fraction of lysed cells would be higher in a microaerophilic or anaerobic environment (7).

Spontaneous competence development in S. pneumoniae is induced by the DNA-damaging agent mitomycin C and the protein synthesis inhibitors streptomycin and kanamycin (61). Recently it was reported that the competence-promoting effect of streptomycin and kanamycin is due to increased ribosomal error rates in cells treated with these antibiotics (71). The finding that natural transformation in S. pneumoniae is induced in response to an increased error rate during protein synthesis strongly indicates that exogenous DNA is taken up by competent pneumococci in order to repair underlying genetic damage. Only DNA from strains or species closely related to the recipient will function as a template for such recombinational repair. This fits very well with the observed properties of fratricins.

A completely different mechanism that discriminates between homologous and foreign DNAs has evolved in naturally transformable members of the Gram-negative families Neisseriaceae and Pasteurellaceae (69, 74). When exposed to a mixture of foreign and homologous DNAs, Neisseria spp. and Haemophilus influenzae preferentially take up homologous DNA. The DNA uptake apparatus of these species recognize 10- to 12-bp sequence motifs that are dispersed throughout their genomes. Competent streptococci, on the other hand, will take up DNA from any source equally well. Most streptococcal species live in multispecies biofilms in the upper respiratory tracts of humans and other mammals. Streptococci that develop the competent state in this environment will undoubtedly run the risk of taking up unrelated DNA that is useless for genome maintenance and repair. Competent streptococci seem to have solved this problem by secreting a fratricin that kills and lyses closely related neighbors in the biofilm, thereby increasing their chances of picking up homologous DNA.

FRATRICIDE AND BIOFILM FORMATION

Streptococci are typically found in multispecies biofilms on mucosal surfaces in humans and animals. Bacteria in such biofilms are embedded in an extracellular matrix consisting of polymeric substances. Extracellular polysaccharides have been considered to be the principal component of the extracellular matrix, but recently its structure has been found to be more complex. The composition of the matrix depends on the microbial cells present and the physicochemical environment in which the biofilm is located. In addition to polysaccharides, the matrix contains various amounts of lipids, proteins, lipopolysaccharides, glycolipids, and nucleic acids (26). Initially, eDNA was not considered an important structural component of biofilms but was rather seen as residual material from disintegrated dead cells. The importance of eDNA was first demonstrated by Whitchurch et al. (78). They found that addition of DNase I to the culture medium strongly inhibited the formation of Pseudomonas aeruginosa biofilms. Similar findings have subsequently been reported for other bacteria, including streptococci (33, 53, 58, 62, 63). Although some eDNA might be excreted by living cells (34), the bulk of eDNA is presumably released from lysed cells. Bacterial lysis could, in principle, be self-inflicted (autolysis) or triggered by enzymes produced by neighboring cells. This raises the question of whether streptococcal fratricins might play a role in biofilm formation by mediating DNA release from susceptible sister cells. Trappetti and coworkers (73) recently reported that a CbpD-deficient mutant of S. pneumoniae D39 was unable to make a biofilm when grown in C⁺Y medium supplemented with 50 μM Fe(III), suggesting that fratricide has an important role in biofilm formation. However, the finding that CbpD-mediated release of eDNA stimulates the formation of monospecies biofilms in the laboratory does not necessarily imply that this mechanism plays an import role under natural conditions. In the multispecies biofilms that constitute the natural habitat of streptococci, different release mechanisms and hundreds of species will contribute to the common eDNA pool. Considering that fratricide is coregulated with natural transformation, it is unlikely that its primary role is to provide eDNA for the biofilm matrix. The fact that the matrix of multispecies bio-
films contains a lot of foreign DNA that, in general, is useless for DNA repair purposes highlights the need for a mechanism that can give competent streptococci access to homologous DNA. In our opinion, fratricide represents this mechanism. In all likelihood, more DNA is released from target cells than is taken up by competent recipient cells. Thus, it is reasonable to assume that fratricide also contributes to the mechanical stability of biofilms.

CONCLUDING REMARKS

Most of the data discussed in this minireview support the theory that streptococci produce fratricins to increase their chances of taking up homologous DNA during the competence period. However, more research is needed to settle this issue. Several important aspects of the pneumococcal fratricide mechanism, which is by far the best-characterized system, are still poorly understood. The exact bond split by CbpD-Sp has not been identified, and the precise target structure recognized by its SH3b domains is still unknown. The mechanism by which ComM protects pneumococci against their own fratricins, an important task for the future is to unravel how competent streptococci protect themselves and their target range.

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REFERENCES

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