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Research Focus

# Quorum sensing in streptococcal biofilm formation

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**Bacteria in their natural ecosystems preferentially grow as polysaccharide-encased biofilms attached to surfaces. Although quorum-sensing (QS) systems directing the 'biofilm phenotype' have been extensively described in Gram-negative bacteria, there is little understanding of the importance of these systems in Gram-positive biofilm formation. Streptococci are a diverse group of Gram-positive bacteria that colonize epithelial, mucosal and tooth surfaces of humans. In several streptococci, competence-stimulating peptide (CSP)-mediated QS has been connected with competence development for genetic transformation. Recent work, especially with bacteria that inhabit the biofilm of dental plaque, has linked CSP stimuli to other cell-density adaptations, such as biofilm formation.**

## Introduction

Biofilm (see Glossary) formation in bacteria is one example of group behavior that is coordinated through a quorum-sensing (QS) system. In the biofilm mode of growth, microorganisms exhibit increased resistance to antimicrobial compounds, environmental stresses and host immune defense mechanisms [1]. Although the role of QS systems and signals in biofilm formation has been well characterized in several Gram-negative organisms, there are relatively few studies that demonstrate their involvement in Gram-positive biofilms. Petersen *et al.* [2] recently reported that a QS signal, termed competence-stimulating peptide (CSP), involved in the development of competence for natural transformation in the Gram-positive bacterium *Streptococcus intermedius*, also favors biofilm formation. The addition of synthetic CSP not only induced competence when cells were exposed to a transformable plasmid but it also significantly enhanced biofilm formation [2]. Intriguingly, Petersen *et al.* [2] also showed a tenfold increase in the proportion of competent cells in biofilms compared with planktonic cell fractions. This article, combined with reports of other streptococcal species responding to CSP, provides new insight into the CSP stimulus, extending it from competence development to other cell-density phenotypes, including biofilm formation.

## Quorum sensing in streptococci

In Gram-negative bacteria, a large number of QS systems have acyl homoserine lactone molecules as signals that activate a transcriptional regulatory protein [3]. By contrast, QS systems in Gram-positive bacteria typically use secreted peptides (often referred to as pheromones) as signal molecules and a two-component regulatory system (composed of a membrane-bound histidine kinase receptor and an intracellular response regulator) to detect the peptide and trigger the required changes in gene expression [4].

In *Streptococcus pneumoniae*, a CSP-QS system mediating competence induction and the transformation process has been reviewed [5] (Figure 1). This CSP-induced mechanism, along with many of its underlying genes, has been identified in other streptococcal species including various strains of the mitis (including *S. pneumoniae* and *Streptococcus gordonii*), anginosus (including *S. intermedius*) and mutans (including *Streptococcus mutans*) groups of streptococci [6,7]. Natural transformation is thought to provide a selective advantage to bacteria by enabling the recipient to acquire novel genes, such as antibiotic-resistance elements and other virulence determinants, from the DNA of donor cells [7]. This phenomenon was first observed in 1928 in a classic study by Griffith who discovered that DNA extracted from *S. pneumoniae* isolated from diseased mice could transfer a virulence factor (capsule), observed as a smooth colony phenotype, to a rough avirulent recipient strain [8].

In *S. pneumoniae*, where studies in competence are most advanced, CSP has been shown to slowly accumulate in the surrounding medium until a threshold concentration is reached, triggering competence development throughout the culture. At the genetic level, CSP interaction with its receptor (ComD) initiates a cascade of four temporally distinct transcription profiles: early, late and delayed gene induction and gene repression [9]. Early-competence genes encode proteins that comprise the cell–cell signaling apparatus, including the CSP precursor (encoded by *comC*), its histidine kinase receptor (*comD*), a cognate response regulator (*comE*), and an alternate sigma factor (*comX*) [10,11] (Figure 1). As part of the early competence wave and controlled by ComE, ComX is a key

### Glossary

**Biofilm:** an organised community of surface-adherent microorganisms embedded in an exopolysaccharide matrix.
**Genetic competence:** a transient physiological state that involves the activation of a specific subset of genes that encode the DNA uptake machinery and recombination proteins that facilitate bacteria to undergo natural transformation.
**Quorum-sensing (QS):** bacterial intercellular communication mechanism that controls gene expression in response to population density.
**Natural transformation:** binding and transport of exogenous DNA from the surrounding environment that is subsequently integrated into the host cell genome by homologous recombination (if donor DNA is a chromosomal DNA fragment) or internalized into the cell but not integrated into the genome (if donor DNA is a circular plasmid carrying no homologous DNA sequence).

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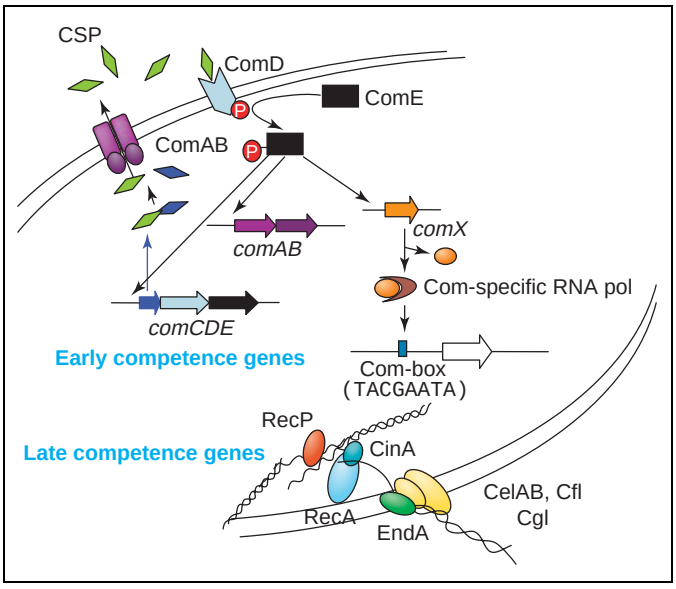


**Figure 1.** The induction of genetic competence in *Streptococcus pneumoniae* is regulated by a competence-stimulating peptide (CSP)-mediated quorum-sensing (QS) system (reviewed in Ref. [5]). This system involves the expression of early gene products encoded by the *comAB* and *comCDE* operons. The *comAB* operon encodes an ATP-binding cassette transporter (ComA; purple rectangle) and an accessory protein (ComB; purple circles) involved in the processing and export of CSP (green diamonds). The *comCDE* operon encodes the CSP precursor (ComC; green and blue diamonds), a histidine kinase CSP sensor (ComD; pale blue chevron), and its cognate response regulator (ComE; black rectangle) that upregulates transcription of *comAB*, *comCDE* and *comX* genes. ComX (brown oval), an alternate sigma factor, together with RNA polymerase (dark brown moon) triggers transcription waves of late competence, delayed gene induction and gene repression [9] by recognizing a *com*-box (also referred to as *cin*-box; blue rectangle) consensus sequence (TACGAATA) in their promoter regions [12]. The delayed and repressed class encodes stress-related functions and protein synthesis (not shown). CSP-QS initiates competence through the activity of ComX inducing transcription of the late-competence genes, mainly involved in DNA uptake and integration into the host cell genome [27]. Several of these late competence-specific operons include DNA uptake apparatus proteins (e.g. CelAB, CfI and CgI; yellow ovals), DNA processing (e.g. EndA; green oval), colligrin (CinA; blue oval) and DNA recombination proteins (e.g. RecA and RecP; light blue and brown ovals). Abbreviations: pol, polymerase; P in red circle, phosphoryl group. Adapted, with permission, from Ref. [18].

regulator that induces later waves of transcription, many genes playing important roles in DNA uptake and integration of extracellular DNA (late-competence genes), stress-related functions (delayed class) and protein synthesis (repressed class) [9,12]. *S. pneumoniae* ComX also regulates a competence-induced release mechanism that initiates cell lysis and release of DNA from a sub-fraction of the bacterial population to serve as donor DNA for genetic exchange [13].

Håvarstein *et al.* [6] found that distinct species of streptococci from the anginosus group commonly encode and respond to identical CSPs, thereby belonging to the same 'pherotype'. In other groups, the CSPs are most often species-specific and in several instances strain-specific. This discovery prompted the use of synthetic, exogenously supplied CSPs that enabled investigators to genetically manipulate and study the molecular biology of specific species or strains of streptococci [14,15].

For several species that were examined in the mitis and anginosus groups of streptococci, the genes encoding the CSP, histidine kinase receptor and the cognate response regulator are organized in a single operon (i.e. *comCDE*) [6]. However in *S. mutans*, an oral bacterium associated with dental caries, *comC* has a distinct promoter and is divergently arranged from the *comDE* operon [15]. Inactivation of the genes encoding the histidine kinase or the response regulator results in complete abolishment of competence for *S. pneumoniae* [11], whereas competence for *S. mutans* is reduced [15]. Furthermore, in *S. mutans* CSP-stimulated competence affects a small subset of cells [16], whereas in *S. pneumoniae* CSP stimulates competence development in most of the exponentially growing cell culture [17]. These studies suggested that the mechanisms of competence and QS regulation might vary among the different streptococcal species [18].

## Evidence for the existence of other CSP-regulated pathways in streptococci

In streptococci, CSP signal also appears to be an effector of multiple genetic pathways, including biofilm formation, acid tolerance, bacteriocin production and virulence [9,18–20]. Transcriptome analysis by Peterson *et al.* [9] during *S. pneumoniae* competence development has shown that among the 124 CSP-inducible genes, only 23 are required for transformation, whereas the vast majority are individually dispensable for transformation under laboratory conditions. Although some of the CSP-induced genes might be important for efficient gene exchange under natural conditions, many of these gene products probably aid in other cell-density adaptive functions; for instance, several CSP-induced genes have stress-related roles or participate in the synthesis and transport of bacteriocin-like proteins [21]. Moreover, an *S. pneumoniae comD* mutant showed reduced virulence in murine models of pathogenesis, indicating that some CSP-regulated genes could have important roles in virulence [19].

The first report to suggest that a CSP-QS system operates in Gram-positive biofilm formation involved the oral bacterium *S. gordonii*. In this organism, a transposon mutation in the *comD* gene resulted in a phenotype that was defective in biofilm formation [22]. Further evidence of an expanded role for CSP was obtained with *S. mutans*, whereby inactivation of any component of its *comCDE* pathway resulted in a phenotype that was both competence- and biofilm-defective [15,23] and had a diminished acid-tolerance response [20]. The study by Petersen *et al.* [9] used an alternative approach for the comparison of wild-type and Com mutant strains by directly examining the effect of CSP on *S. intermedius* biofilm formation [2]; biofilm formation was enhanced in the presence of CSP without affecting the bacterium's growth rate. The use of the different strategies has clearly established the involvement of the CSP-QS system in the process of biofilm formation in three separate species of biofilm-forming streptococci. Moreover, in *S. mutans* and *S. intermedius* CSP appears to influence the initial stages of the biofilm mode of growth (i.e. cellular accumulation on surfaces) rather than the latter maturation step, because *S. mutans comD* or *comE* mutants adhered less to surfaces [23] and *S. intermedius* CSP promoted the early buildup of biofilm cells [2].

Perhaps the most fascinating finding in *S. intermedius* [2] and *S. mutans* [15] was that cells growing in biofilms were able to incorporate foreign DNA much more efficiently

(at least 10-fold higher) than their planktonic counterparts. In a time-course study, Li *et al.* [15] determined that the higher transformation efficiency in *S. mutans* biofilm cells compared with planktonic cells lasts from 8 to 24 hours of growth, although biofilm transformability slowly decreases as the biofilm further ages. Additionally, the *comCDE* QS system also appears to be transcriptionally upregulated when *S. gordonii* [24] or *S. mutans* [15,16,23] cells are living in actively growing biofilms. These findings clearly suggest that the biofilm formation in transformable streptococci appears to favor the optimal function of streptococcal CSP cell–cell signaling to activate genetic competence and facilitate genetic exchange [7,16]. Although transformation has been extensively studied in planktonic streptococci, where cultures become transiently competent (20–40 minutes) after reaching a critical cell density [15,21], there has been little progress in understanding gene exchange under biofilm conditions. It is probable that the high cell-density biofilm environment, in addition to providing cells with an abundant extracellular gene pool, establishes gradients of CSP signal and provides other environmental factors (e.g. nutrients, oxygen availability and pH) that sustain localized clusters of cells with an extended competence window [18]. Confocal microscopic examination of *S. mutans* GFP-linked *comX* activity demonstrated that there was a spatial relationship with *comX* activation; cells in the denser areas of the biofilm had an increased level of *comX* activity and probably represented a sub-population of cells that were genetically competent [16].

## Future perspective

Although Li *et al.* [23] and Petersen *et al.* [2] demonstrated that the CSP-QS system was connected to the ability of *S. mutans* and *S. intermedius* to form biofilms, the exact molecular and biochemical details involved in CSP-QS regulation of the 'wild-type biofilm phenotype' needs to be investigated. A functional genomic approach, such as microarray or two-dimensional gel electrophoresis (2DE) proteomic analysis of streptococcal wild-type and *comC* (CSP) mutant biofilms grown in the presence and absence of CSP, would prove especially beneficial in elucidating the underlying mechanisms of CSP influence on biofilm formation. The recent *S. pneumoniae* microarray results identified CSP-induced metabolic and stress-response genes that might play a role in streptococcal adaptation to a higher cell density during biofilm formation [9]. Moreover, environmental and metabolic factors that influence biofilm formation and CSP-QS responses should be examined. This connection was recently highlighted in *Staphylococcus aureus* where the precise role of the *agr* signaling system involved in biofilm formation was found to be dependent upon the environmental conditions under which the biofilm is grown [25]. Furthermore, clear evidence of the role of CSP in other cell-density phenotypes (i.e. bacteriocin production or virulence) of transformable streptococci, particularly in *S. pneumoniae*, *S. intermedius* and oral streptococci, remains to be established.

Because of their role in coordinating biofilm formation and activating virulence factors in many Gram-negative and Gram-positive bacteria, QS systems have emerged as an enticing target for fighting biofilm infections. Thus, inhibition of the streptococcal CSP-mediated pathway appears a feasible method to interfere with biofilm formation, rendering streptococci more vulnerable to antimicrobial compounds and the host immune response. One approach might involve the synthesis of a CSP analog to act as a specific competitive inhibitor of the interaction between CSP and the histidine kinase receptor, hindering induction of the 'biofilm phenotype'. For example, Otto *et al.* [26] recently demonstrated that modified forms of the AgrD signal peptides from *Staphylococcus epidermidis* could inhibit the *agr* signaling system of *S. aureus*, modulating its virulence. Future antimicrobials based on QS interference should have a high specificity against target organisms, leaving beneficial commensal bacteria unharmed during therapy, with a side benefit of a minimal likelihood of antibiotic resistance being transmitted between species. With the inevitable decline of the effectiveness of currently used antimicrobials these novel approaches should be seriously considered.

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