CHAPTER 12

Quorum Sensing and Biofilm Formation by *Streptococcus mutans*

Dilani Senadheera and Dennis G. Cvitkovitch*

Abstract

Streptococcus mutans is the primary causative agent involved in dental caries in humans. Among important virulence factors of this pathogen, its ability to form and sustain a polysaccharide-encased biofilm (commonly called dental plaque) is vital not only to its survival and persistence in the oral cavity, but also for its pathogenicity as well. This chapter focuses on the *S. mutans'* biofilm phenotype and how this mode of growth is regulated by its density-dependent quorum sensing (QS) system primarily comprised of the Competence Stimulating Peptide (CSP) and the ComD/ComE two-component signal transduction system. In addition to biofilm formation, the CSP-mediated QS system in *S. mutans* also affects its acidogenicity, aciduricity, genetic transformation and bacteriocin production. Interestingly, it has also been discovered that these properties are optimally expressed in cells derived from a biofilm as opposed to a free-floating planktonic mode of growth. Hence, strategies targeting *S. mutans'* QS system to attenuate biofilm formation and/or virulence are currently being used to develop therapeutic or preventative measures against dental caries. Recently, it was discovered that the addition of CSP in large concentrations (relative to amounts used for normal competence development) resulted in growth arrest and eventual cell death, thus paving way for CSP-mediated targeted killing of *S. mutans*. In addition to the QS system, effects of other two-component signal transduction systems on the biofilm phenotype of *S. mutans* are also discussed.

Introduction

In nature, bacteria live in diverse habitats that are often subjected to various environmental fluxes. The ability to detect and adapt their cell physiology or behavior to signals reflecting such changes can ultimately determine the survival and persistence of these organisms. The two-component signal transduction paradigm, illustrated by the chapters in this book, facilitates this adaptive response via a sophisticated sensor and responder. More specifically, a particular stimulus or a combination of stimuli can autophosphorylate a membrane-located histidine kinase sensor, which can then convey this message into the cell by transferring the phosphate group to a responder protein (response regulator).[1,2] The adaptive phenotype is elicited when the resulting phosphorylation triggers a conformational change in the response regulator prompting its binding to specific DNA promoter regions, thereby rendering transcriptional regulation of genes under their control. In recent years, the availability of complete bacterial genomes has facilitated the discovery of novel two-component signal transduction systems, which in combination with effective genetic tools has vastly enhanced our understanding of how bacteria sense and respond

*Corresponding Author: Dennis G. Cvitkovitch—Oral Microbiology, Faculty of Dentistry, University of Toronto, 124 Edward St., Toronto, Ontario M5G 1G6, Canada. Email: d.cvitkovitch@dentistry.utoronto.ca

Bacterial Signal Transduction: Networks and Drug Targets, edited by Ryutaro Utsumi.

to various signals. From a clinical standpoint, these systems provide us with potential targets that can be manipulated to combat pathogenic infections.

The quorum sensing (QS) phenomenon, which enables bacteria to alter their gene expression when a critical density of the cell population is reached, is one of the most fascinating behaviors observed utilizing signal transduction.[3-8] Ubiquitous in both Gram-negative and Gram-positive bacteria, QS is induced by chemical messengers (autoinducer molecules or pheromones) that are released into the environment and increased in concentration proportionate to a growing population. Once the density reaches a particular threshold, these signal molecules can trigger an expression cascade, which can alter the cell populations' physiology or behavior as a collective unit instead of an individual entity. Various physiological activities controlled by this cell-cell communication circuitry include antibiotic production, competence development, sporulation, biofilm differentiation, conjugation and bioluminescence in bacteria.[6,9-17] Such highly coordinated group behavior can have profound implications on the survival as well as the pathogenicity of a bacterial population. In addition to mounting stress responses and coping mechanisms under high cell density, it may be advantageous for these organisms to synchronize the release of toxins and other antigenic virulence factors with cell density and thus overwhelm the host immune system with the sudden unanimous release of virulence factors. Accumulating evidence of infectious models conducted by using animals harboring mutant bacterial strains defective in one or more components of their respective QS systems have shown reduced or attenuated virulence.[6,18]

In this ensuing chapter, we will discuss how *Streptococcus mutans*—a primary etiologic agent responsible for human dental caries as well as endocarditis, utilizes its QS signaling system to regulate biofilm formation. Biofilms are surface-attached microbial communities that are protected by a self-generated organic polymer matrix.[19-24] By resorting to a biofilm lifestyle, *S. mutans* can modulate various physiological and physical properties that are beneficial to its growth, survival and persistence in the oral cavity. For example, the biofilm matrix can act as a diffusion barrier and limit the penetration of antimicrobials to the innermost cells. Also, biofilm cells have a transcriptional profile that is markedly distinct from their planktonic counterpart, likely giving rise to their inherently resilient or resistant phenotype that is widely documented in the scientific literature.[20,25-30] On a different note, it has been shown that *S. mutans* cells grown as a biofilm have a dramatic increase in genetic competence and are highly transformable by exogenous DNA.[38] They also exhibit an increased ability to tolerate acid challenges.[13,31] Much to the fascination of researchers in this field, it was recently discovered that the addition of the biologically active synthetic autoinducer QS peptide pheromone in excess of concentration required for the induction of genetic transformation caused growth arrest and eventually cell death, followed by disruption of the biofilm.[32] This finding was an exciting discovery for the scientific community investigating the possibility of targeting *S. mutans* to control its pathogenicity.

Virulence Properties of *S. mutans*

Streptococcus mutans is a well known bacterium in the fields of oral microbiology and clinical dentistry as the etiologic agent of dental caries in humans. *S. mutans* was first described as small, chained coccobacilli by J. K. Clark in 1924, although its relationship with caries was clearly distinguished only in the 1960s following a series of experiments by Fitzgerald and Keyes, who demonstrated its capability to induce dental caries in rats and hamsters.[33] However, a link between *S. mutans* and human dental caries was only established after a series of longitudinal studies that showed a statistically significant increase in *S. mutans*' counts in teeth that were destined to develop carious lesions in humans.[34-38] After recognizing the association of *S. mutans* with human dental caries, decades of research has focused not only on identifying its virulence traits, but also on understanding how these factors are regulated in the plaque biofilm to cause disease. A growing evidence of literature has highlighted three virulence traits of *S. mutans* as being vital to the initiation and progression of caries. These include: (1) the ability to metabolize dietary carbohydrates and produce lactic acid (acidogenicity), (2) ability to grow and survive in a low pH environment (aciduricity) and (3) ability to utilize dietary sugars to produce glucan polymers and form the plaque biofilm.[39-42] Of the first

two factors, acidogenicity or the release of acid into its growth environment results in corroding the calcium-phosphate matrix of the tooth structure, whereas high aciduricity of *S. mutans* enables it to survive and further utilize sugars for metabolic activities under low pH that is usually detrimental to a large number of plaque organisms. The third attribute, which is the ability to form a biofilm offers *S. mutans*' protection from transient environmental changes and mechanical forces, thereby facilitating its growth and survival in the oral cavity. In fact, *S. mutans* is so attuned to the biofilm lifestyle that it cannot be found in the mouths of people without teeth or dentures for it to adhere to. The carbohydrate polymer matrix in the biofilm also serves as an excellent food reserve from which *S. mutans* and other bacteria can derive nutrients by digesting these organic polymers. Interestingly, in *S. mutans*, aciduricity and biofilm formation are controlled by a peptide-induced QS system,[13,43] which we will discuss in the following section.

Quorum Sensing System in *S. mutans*

Identification of the Pneumococcal Quorum Sensing System

The discovery of the QS network in *S. mutans* was largely dependent on information derived from its closely related species, *Streptococcus pneumoniae*, which comprises the best-characterized cell-cell communication circuitry among members of the genus *Streptococcus*. In *S. pneumoniae*, QS was believed to function mainly to acquire and incorporate foreign DNA from the environment by induction of a physiological state known as genetic competence. In this bacterium, the link between cell population density and transformation was first discovered in the 1960s by Pakula and Walczak (1963) and Tomasz and Hotchkiss (1964).[44,45] Upon noticing that the induction of genetic competence took place only at a particular cell population density, it was also discovered that supplementing cell-free supernatant derived by filtering a competent culture was capable of inducing competence in otherwise noncompetent cells. It was later determined that the competent state was induced by a protein-like "activator" compound present in the supernatant of the competent cultures.[44,46] Later this peptide was identified as a 17-residue, cationic peptide and was referred to as the competence stimulating peptide (CSP) due to its role in genetic competence development in *S. pneumoniae*.[47,48] By using the reverse translated amino acid sequence of the CSP, the *comC* precursor peptide gene (*com* for competence) and its contiguous genes were identified.[47,49-51] Interestingly, the *comC* was part of the *comCDE* tricistronic operon, in which the latter genes encoded a membrane-bound histidine kinase sensor protein (ComD) and its cognate respond regulator (ComE).[52,53]

Competence Stimulating Peptide

The QS circuit in *S. mutans* was discovered by searching its genome using the *comCDE* sequence of *S. pneumoniae*. Despite the high homology shown by the orthologous genes in *S. mutans*, the orientation of these genes was distinct from that in *S. pneumoniae*: the *comC* was located 59 bp proximal and in the reversed orientation of the complementary strand harboring *comDE*.[31] In addition to *S. pneumoniae*, the organization of these genes in *S. mutans* also differed from those in other streptococci including *Streptococcus mitis*, *Streptococcus oralis*, *Streptococcus gordonii* and *Streptococcus sanguinis*.[54] In these bacteria, the *comC*, *comD* and *comE* genes are organized as an operon starting with *comC* and followed by *comD* and *comE* genes in the 5' to 3' direction.

The deduced primary translational product of *comC*, which was derived by using its consensus nucleotide sequences from various *S. mutans* strains, was identified as a 46-amino acid cationic peptide, whose C-terminus harbored the biologically active secreted CSP, 21-aa in length.[31] As in the case of pneumococci, posttranslational processing was required for the maturation of the propeptide by cleaving the Gly-Gly site located at -1 and -2 positions (relative to the cleavage site) in the N-terminus of the precursor CSP. Interestingly, the biological activity of the mature synthetic CSP was retained when added to growing cultures as judged by the increased frequency in transformation.[31] Hence, this finding provided researchers with a novel tool to use synthetic, exogenously supplied CSP to simulate population growth in *S. mutans*' cultures in the laboratory, as well as further investigate the physiological properties controlled by CSP-induced QS in

S. mutans. The ability to use synthetic CSPs for QS experiments was demonstrated prior to this study by Håvarstein et al who showed that distinct species of streptococci from the anginosus group commonly encoded and responded to identical CSPs, thereby belonging to the same 'pherotype'.[55] In contrast, it was discovered that CSPs from other groups were most often species-specific and in some cases strain-specific.

In a recent study, Syvitski et al[56] investigated the structure-function relationship of the *S. mutans* signaling peptide based on three-dimensional structures of CSP derived from the UA159 wild-type strain and a C-terminally truncated peptide (TPC3) from the JH1005 strain, which was defective in genetic competence development. By synthesizing a series of peptides that harbored aa-substitutions or aa-deletions and by using them in competitive inhibition assays, these researchers showed that the C-terminal structural motif comprising of polar-hydrophobic charged residues was crucial for QS activation, whereas the core alpha-helical structure present in the mature peptide was important for receptor binding. It was demonstrated that the lack of 3 or more C-terminal peptides resulted in noncompetent cells and that these mutant peptides, at a higher concentration, were able bind and competitively inhibit and eventually abolish the activation of the CSP-induced QS pathway in *S. mutans*.

ComDE Signaling Pathway

In *S. mutans*, the QS pathway encompasses at least two genetic loci—*comCDE* and the *comAB*.[31] While *comC* encodes the precursor CSP, the *comDE* genes encode a two-component signal transduction system comprising of a membrane-bound histidine kinase (ComD) and its cognate response regulator (ComE), respectively. The *comA* and *comB* genes encode the secretion apparatus necessary for processing and export of the signaling molecule. More specifically, the ComA consists of an ATP-binding cassette transporter that utilizes ComB as an accessory protein for processing of the precursor CSP. Based on the QS mechanism of *S. pneumoniae*, a model for QS in *S. mutans* has been proposed[31] (Fig. 1). It is believed that when mature CSP reaches a threshold concentration, it is detected by the ComD receptor, which undergoes autophosphoryaltion at a conserved histidine residue. Consequently, the phosphorelay of ComD to its responder protein, results in its activation, thus transducing the density-dependent message into a cellular response by altering the transcription of *comAB, comC* and *comDE*, as well as that of an alternative sigma-factor (designated *comX*) that can regulate the so-called "late genes".[57] Recently, it has been shown that in the absence or in the presence of low CSP concentrations, unphosphorylated ComE can repress the transcription of *comC*, whereas under high CSP concentration phosphorylated ComE can increase the comC transcription via a de-repression mechanism.[58] The ComE-dependent activation of the late-phase is one of four temporally distinct transcription profiles observed in response to CSP in *S. pneumoniae*.[59] These include the early, late, delayed gene induction and gene repression profiles that are yet to be identified in *S. mutans*. Of these transcriptional profiles, the early genes encode proteins necessary for CSP production, export and recognition and include the *comAB, comCDE* and *comX* genes whose activation is likely dependent on the phosphorylated state of ComE. In the pneumococcal competence development model, the ComE-binding site consensus sequence includes aCAtTTca/gG-N_{12}-ACAt/gTTgAG.[60] Interestingly, activated ComX together with RNA polymerase (which are stabilized by ComW) can recognize and bind to a conserved promoter sequence of downstream target genes under their control. In *S. pneumoniae*, this consensus sequence (TACGAATA) is known as the com-box or cin-box and are known to control genes important for DNA processing, uptake and recombination.[61-63] Interestingly, CSP-induced ComX activation also promotes the cell-lysis and release of DNA from a sub-fraction of the bacterial population.[64,65] This observation is not surprising since, by regulating the availability of donor DNA in concert with genetic competence, these bacteria ensure that the energy and resources utilized by cells for genetic transformation is worthwhile. A similar phenomenon of DNA release via QS was observed in *S. mutans*,[32] which will be discussed in the following sections along with other phenotypes controlled by the CSP in this bacterium.

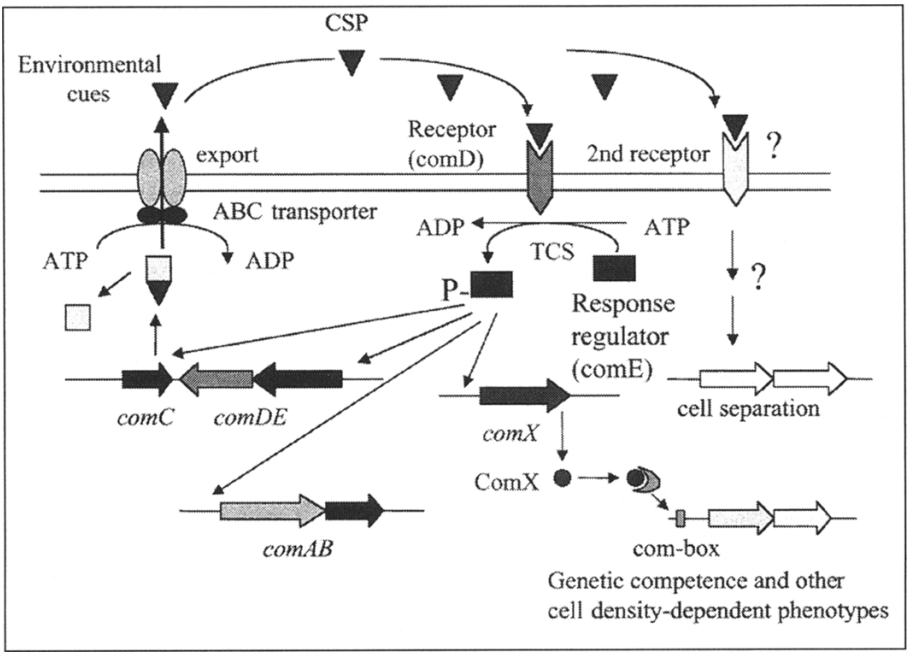

Figure 1. Schematic representation of the putative molecular mechanism of the QS system in *S. mutans*. From Li YH et al. J Bacteriol 2002:2699-708;[43] with permission of the American Society for Microbiology.

Quorum Sensing and Biofilm Formation in *S. mutans*

Activation of the QS system in *S. mutans* is known to regulate several phenotypes: biofilm formation, competence induction, acid tolerance response (ATR) and bacteriocin production.[13,31,43,66] Interestingly, evidence suggests that transformability and ATR function optimally when *S. mutans* is grown as a biofilm in contrast to free-living planktonic cells.[13,31] In this section, we will give prominence to discussing the CSP-mediated biofilm phenotype and how the QS pathway can be manipulated to control the formation of dental biofilms.

In Gram-positive bacteria, the first report to establish a link between QS and biofilm formation came from an investigation by Loo et al involving *S. gordonii*.[14] This bacterium is a pioneer organism in the oral microflora that initiates the formation of dental plaque. In an attempt to identify various genetic factors involved in biofilm formation in the *S. gordonii* Challis strain, these researchers used Tn*916* transposon mutagenesis to screen for mutants defective in biofilm formation using an in vitro biofilm assay. It was revealed that one of the mutants, which had a transposon inserted in the *comD* signal peptide histidine kinase sensor gene formed defective biofilms that lacked the three-dimensional structure present in wild-type biofilms, thus linking QS to biofilm formation.

When the genetic components of the *S. mutans*' QS system were first identified, it was observed that the genetic transformability was 10- to 600-fold higher in cells derived from biofilms, compared with those obtained from planktonic cultures.[31] Hence, it was hypothesized that in addition to competence development, this system might also be involved in biofilm formation. To test this assumption, mutants deficient in the *comC*, *comD*, *comE* and *comX*, as well as the entire *comCDE* operon were constructed and assayed for their ability to initiate biofilm formation.[43] Interestingly, compared with the wild-type, all mutants formed biofilms that lacked the architectural integrity of the wild-type biofilm, whereas the *comD*-, *comE*- and *comX*-deficient mutants formed biofilms

with reduced biomass. Notably, only biofilms formed by the *comC* mutant and not those formed by the *comD, comE* or *comX* mutants were restored to the wild-type architecture by complementing with synthetic CSP or a plasmid containing the wild-type *comC* (Fig. 2). This discrepancy was likely caused by the involvement of multiple signal transduction pathways in detecting and/or responding to CSP. However, the identity and the mode of regulation of a second putative CSP-responsive system are yet to be established. Moreover, expression analysis of the *comCDE* genes in *S. mutans* and *S. gordonii* showed that they were upregulated in the biofilm mode of growth relative to their planktonic counterpart further supporting the QS-biofilm link.[14,43]

Recently, in an attempt to understand the molecular basis of biofilm regulation by the CSP-dependent QS system, the expression of several biofilm-associated genes were tested using 18-h biofilms derived from *S. mutans* wild-type UA159 and its isogenic mutant derivatives deficient in the *comC, comD* or *comE* genes.[67] Expression analysis showed that the ComD/ComE signal transduction system had a positive regulatory effect on the expression of these genes, which include glucosyltransferase B/C/D (*gtfB/C/D*), fructosyltransferase (*ftf*) and glucan-binding protein B (*gbpB*). Of these, the glucosyltransferases utlilze the glucan moiety of the sucrose molecule to produce insoluble (GtfB/C) and soluble (GtfD) glucan polymers that promote plaque formation. In a different study, it was discovered that *gtfB/C* expression were significantly upregulated in *S. mutans* UA159 when supplemented with CSP relative to the no-CSP control in a ComE-dependent manner.[68] Interestingly, since previous studies have linked these genes with cariogenicity in rat models, their regulation by the QS system in *S. mutans* is indeed insightful and could potentially lead to the design of a new drug target that may affect QS-mediated biofilm formation.

In a different study, Petersen et al investigated the specificity of the CSP effect on biofilm formation utilizing *S. mutans* and *Streptococcus intermedius*.[69] They observed that each species only responded to its native CSP and showed no biofilm response when exposed to the nonnative peptide. For example *S. mutans* wild-type LT-11 responded to its native peptide by enhancing biofilm formation but was unaffected by the signal peptide produced by *S. intermedius*. This kind of specificity displayed by QS peptides can be used to our advantage to specifically target biofilm formation by potentially pathogenic bacteria. In fact, such a technique would be less invasive and possibly have less influence in disrupting the ecological imbalance of the oral microflora by killing particular opportunistic pathogens using antibacterial drugs.

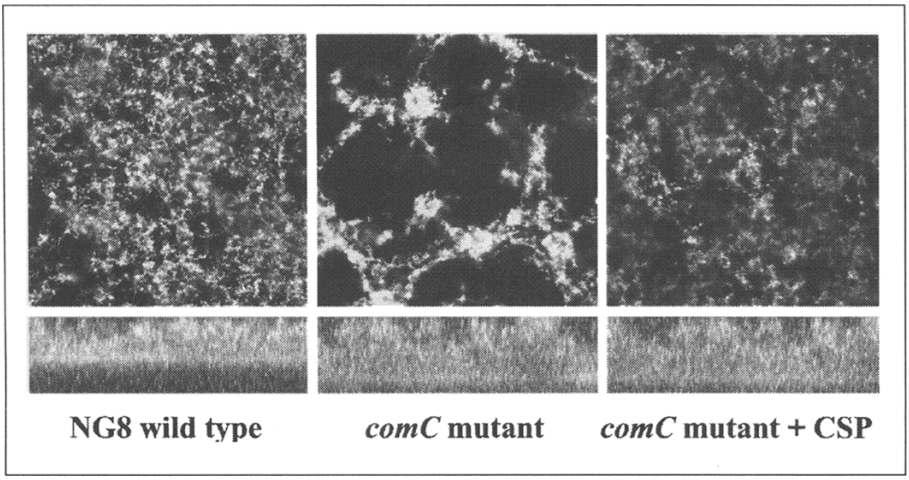

Figure 2. Biofilm formation by ComC mutant and its NG8 parent with and without CSP at 300X magnification using CSLM. Top panel: *xy* plane, lower panel: *xz* panel. From Li YH et al. J Bacteriol 2002:2699-708;[43] with permission of the American Society for Microbiology.

Density-Dependent Production of Bacteriocins: Implications on Survival in Plaque

Recent discoveries pertaining to the QS system in *S. mutans* have demonstrated a link to bacteriocin or bacteriocin-like gene expression in this organism. Bacteriocins (mutacins) are ribosomally synthesized proteins, which have antimicrobial activity against closely related bacteria.[70] In *S. mutans*, two types of mutacins have been identified: (1) lantibiotics represented by mutacin I, II and III (2) nonlantibiotics represented by mutacin IV.[71-74] While the former type can kill most of the Gram-positive bacteria, the latter has a narrower spectrum killing mostly streptococci belonging to the sanguinis and mitis types. As a resident in the oral biofilm, the activity of bacteriocins can help *S. mutans* compete for limited nutrients available in its ecological niche. The high genetic diversity observed among different *S. mutans* strain might be a result of the QS-dependent bacteriocin production that is coupled with horizontal gene transfer. Moreover, by synchronizing bacteriocin production with population density, *S. mutans* can not only reduce its competition for food, but also ensure that a high percentage of heterologous DNA is present during genetic competence development. In addition to producing bacteriocins, *S. mutans* was recently shown to produce bacteriocin-immunity proteins (encoded by *bip* and *smbG*), which modulate the sensitivity to antimicrobials in *S. mutans*.[75] In the presence of low antibiotic concentrations, genes encoding these bacteriocin-immunity proteins showed an increased expression, whereas their optimal expression was seen when cells were grown as a biofilm as opposed to planktonic cells.

Quorum Sensing-Dependent Growth Arrest and Cell Death

Based on multiple phenotypes controlled by the *S. mutans* QS system, it seems rational that manipulating QS can possibly reduce its effects on various virulence attributes controlled by this system. Recently, it was demonstrated that the addition of exogenous CSP in excess of the levels necessary for competence induction resulted in the inhibition of *S. mutans* growth.[32] Not surprisingly, this effect was ComDE-dependent and when CSP concentration was further increased, the cells underwent growth arrest and cell death. Mutational analyses suggested that cell death was mediated by ComD/ComE in a ComX-independent manner. More recently, the effect of CSP (0 to 10 μg/ml) on *S. mutans* biofilm architecture was investigated using Scanning Electron microscopy.[68] Supplementing *S. mutans* biofilms with high dose of synthetic CSP (5 or 10 μg/ml) resulted in a high fraction of ruptured and swollen cells that also showed a different cell shape and surface texture. Hence, these observations reveal that the effects of CSP on biofilm formation are multifactorial. It is yet to be determined whether it is possible to selectively control *S. mutans* in a multi-species biofilm by varying the CSP concentration. Moreover, using CSP to control *S. mutans* to modulate virulence obviously warrants further in vivo testing.

Effect of Other Signal Transduction Systems on *S. mutans* Biofilm Formation

In addition to the ComD/ComE system, other two component signal transduction systems in *S. mutans* have been associated with biofilm formation (Fig. 3). Of these, the VicR/VicK system has been shown to control the expression of *gtfB/C/D*, *ftf* and *gbpB* genes.[76] The *vicK* and *vicR* genes encode a putative histidine kinase and putative response regulator, respectively. While the *vicR* serves an essential function in *S. mutans*, mutational analyses using a *vicK*-deletion strain revealed that it affected biofilm architecture, cell growth and sucrose-dependent adhesion in *S. mutans*. Interestingly, although this mutant produced an excess of extracellular polymer, specific pathogen-free rats harboring the *vicK* mutant strain showed no difference in dental caries relative to those that harbored the parent strain. However, more smooth-surface plaque and significantly diminished mutant CFUs were observed relative to the control group. Since it is known that the cariogenicity in these animals are affected by the microbial composition, the diet and the nature of the polysaccharide matrix, it is not surprising that the excess plaque extent observed for the *vicK* mutant did not necessarily result in hypercariogenicity. However, it is noteworthy that without

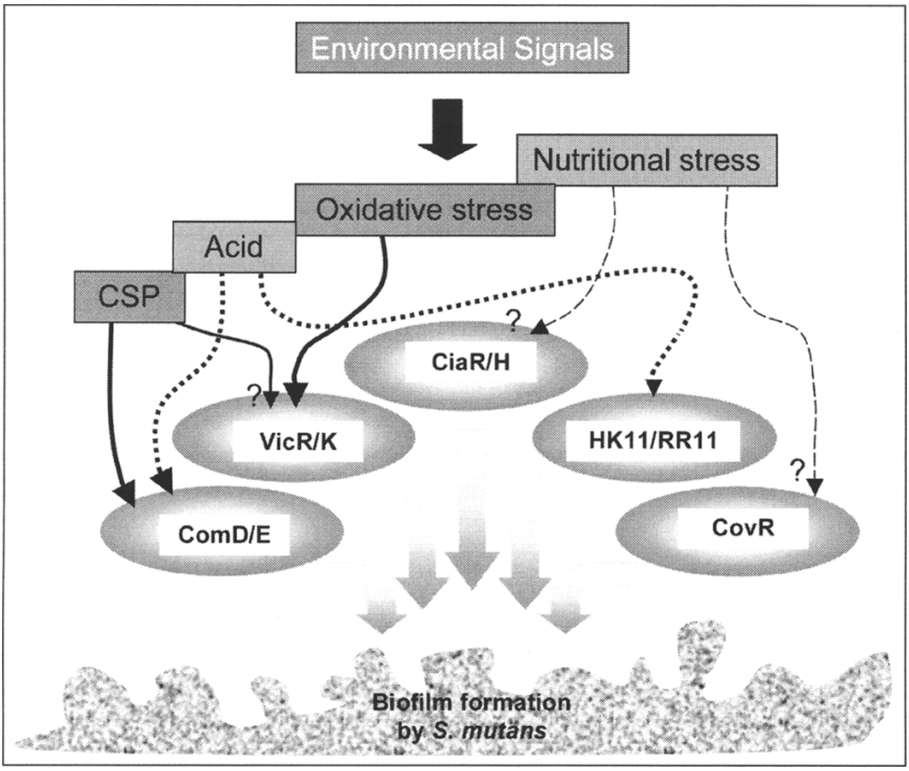

Figure 3. QS-mediated phenotypes in *S. mutans* and two-component signal transduction systems that modulate the biofilm phenotype. From Li YH et al. J Bacteriol 2002:2699-708;[43] with permission of the American Society for Microbiology.

vicK the excess plaque that was generated by the mutant strain was not cariogenic, thus making it interesting to identify the nature and biochemical properties of this polymer.

Studies pertaining to two other signal transduction systems, HK11/RR11[77] and CiaR/CiaH,[78] as well as an orphan response regulator CovR[79,80] have also shown to influence the biofilm phenotype in *S. mutans*. In each signal transduction system, the *hk11/rr11* and the *ciaH/ciaR* genes encode a histidine kinase sensor protein and its cognate response regulator, respectively. The hk11/rr11 system was investigated by Li et al who showed that deletion of *hk11* or *rr11* caused defects in biofilm formation with 50% to 70% reduction in biofilm biomass and resistance to acidic pH.[77] Mutant biofilms observed by Scanning electron microscopy revealed biofilms having a sponge-like architecture with large open areas throughout the biofilm. In a different study, inactivation of either of the *ciaR/ciaH* genes resulted in reduced biofilm biomass, whereas the absence of *ciaH* altered sucrose-dependent biofilm formation.[78] Interestingly, this histidine kinase mutant also showed reduced mutacin production, deficiency in transformability, as well as diminished ability to tolerate stress. Involvement of the CovR mutant (also called GcrR) in biofilm formation was first discovered by Idone et al.[80] These researchers showed that a mutant deficient in *covR* was defective in sucrose-dependent adhesion and was hypocariogenic in a germ-free rat model relative to its parent strain. Consequently, it was discovered by Biswas et al that the CovR negatively regulated the expression of the *gtfB* and *gtfC* genes by directly binding to the promoter region.[79] While these signal transduction systems possibly cross-communicate to regulate biofilm formation in *S. mutans* more studies are warranted to investigate if and how they function in response to cell density and other environmental signals.

Future Perspectives

The prospect of targeting QS systems to control biofilm-mediated infections is receiving a considerable amount of attention. Chemical agents that mimic QS molecules are constantly being evaluated for their ability to 'confuse' the bacteria and hopefully attenuate their ability to cause disease. This is indeed a paradigm shift in the way infections are treated and managed. Current strategies of antimicrobial action are focused on molecules with broad-spectrum indiscriminate activity. The treatment of patients with these compounds often results in massive alteration of the host's microflora often with health affecting consequences (such as ulcerative pseudomembranous colitis). By specifically targeting QS systems of pathogenic bacteria we may hopefully be able to surgically remove the pathogens while leaving the normal flora intact with hopefully little or no detrimental side effects to the host. As we learn more about the subtle differences between bacteria and the way they sense their environments these approaches will very likely become commonplace. The day that a diagnostic lab identifies a pathogen and prescribes a highly specific treatment that has the highest effectiveness with the least likelihood of causing secondary problems is within reach.

References

1. Stock AM, Robinson VL, Goudreau PN. Two-component signal transduction. Annu Rev Biochem 2000:183-215.
2. West AH, Stock AM. Histidine kinases and response regulator proteins in two-component signaling systems. Trends Biochem Sci 2000:369-376.
3. Bassler BL. How bacteria talk to each other: regulation of gene expression by quorum sensing. Curr Opin Microbiol 1999:582-587.
4. Bassler BL. Small talk. Cell-to-cell communication in bacteria. Cell 2002:421-424.
5. Henke JM, Bassler BL. Bacterial social engagements. Trends Cell Biol 2004:648-656.
6. Kleerebezem M, Quadri LE. Peptide pheromone-dependent regulation of antimicrobial peptide production in Gram-positive bacteria: a case of multicellular behavior. Peptides 2001:1579-1596.
7. Kleerebezem M, Quadri LE, Kuipers OP et al. Quorum sensing by peptide pheromones and two-component signal-transduction systems in Gram-positive bacteria. Mol Microbiol 1997:895-904.
8. Suntharalingam P, Cvitkovitch DG. Quorum sensing in streptococcal biofilm formation. Trends Microbiol 2005:3-6.
9. Alloing G, Martin B, Granadel C et al. Development of competence in Streptococcus pneumoniae: pheromone autoinduction and control of quorum sensing by the oligopeptide permease. Mol Microbiol 1998:75-83.
10. De Kievit TR, Iglewski BH. Quorum sensing, gene expression and Pseudomonas biofilms. Methods Enzymol 1999:117-128.
11. Kleerebezem M. Quorum sensing control of lantibiotic production; nisin and subtilin autoregulate their own biosynthesis. Peptides 2004:1405-1414.
12. Lazazzera BA. Quorum sensing and starvation: signals for entry into stationary phase. Curr Opin Microbiol 2000:177-182.
13. Li YH, Hanna MN, Svensäter G et al. Cell density modulates acid adaptation in Streptococcus mutans: implications for survival in biofilms. J Bacteriol 2001:6875-6884.
14. Loo CY, Corliss DA, Ganeshkumar N. Streptococcus gordonii biofilm formation: identification of genes that code for biofilm phenotypes. J Bacteriol 2000:1374-1382.
15. Fuqua WC, Winans SC, Greenberg EP. Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators. J Bacteriol 1994:269-275.
16. Bassler BL, Greenberg EP, Stevens AM. Cross-species induction of luminescence in the quorum-sensing bacterium Vibrio harveyi. J Bacteriol 1997:40,43-45.
17. Dunlap PV. Quorum regulation of luminescence in Vibrio fischeri. J Mol Microbiol Biotechnol 1999:5-12.
18. Finch R, Pritchard D, Bycroft B et al. Quorum sensing: a novel target for anti-infective therapy. J Antimicrob Chemother 1998:569-571.
19. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science 1999:1318-1322.
20. Fux CA, Costerton JW, Stewart PS et al. Survival strategies of infectious biofilms. Trends Microbiol 2005:34-40.
21. Stoodley P, Sauer K, Davies DG et al. Biofilms as complex differentiated communities. Annu Rev Microbiol 2002:187-209.
22. Sutherland I. Biofilm exopolysaccharides: a strong and sticky framework. Microbiology 2001:3-9.
23. Kolenbrander PE. Oral microbial communities: biofilms, interactions and genetic systems. Annu Rev Microbiol 2000:413-437.

24. O'Toole G, Kaplan HB, Kolter R. Biofilm formation as microbial development. Annu Rev Microbiol 2000:49-79.
25. Fux CA, Stoodley P, Hall-Stoodley L et al. Bacterial biofilms: a diagnostic and therapeutic challenge. Expert Rev Anti Infect Ther 2003:667-683.
26. Costerton W, Veeh R, Shirtliff M et al. The application of biofilm science to the study and control of chronic bacterial infections. J Clin Invest 2003:1466-1477.
27. Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. Lancet 2001:135-138.
28. Gilmore KS, Srinivas P, Akins DR et al. Growth, development and gene expression in a persistent Streptococcus gordonii biofilm. Infect Immun 2003:4759-4766.
29. Keren I, Shah D, Spoering A et al. Specialized persister cells and the mechanism of multidrug tolerance in Escherichia coli. J Bacteriol 2004:8172-8180.
30. Donlan RM. Role of biofilms in antimicrobial resistance. Asaio J 2000:S47-52.
31. Li YH, Lau PC, Lee JH, Ellen RP et al. Natural genetic transformation of Streptococcus mutans growing in biofilms. J Bacteriol 2001:897-908.
32. Qi F, Kreth J, Lévesque CM, Kay O et al. Peptide pheromone induced cell death of Streptococcus mutans. FEMS Microbiol Lett 2005:321-326.
33. Tanzer JM. Dental caries is a transmissible infectious disease: the Keyes and Fitzgerald revolution. J Dent Res 1995:1536-1542.
34. Loesche WJ, Straffon LH. Longitudinal investigation of the role of Streptococcus mutans in human fissure decay. Infect Immun 1979:498-507.
35. Masuda N, Tsutsumi N, Sobue S et al. Longitudinal survey of the distribution of various serotypes of Streptococcus mutans in infants. J Clin Microbiol 1979:497-502.
36. Walter RG. A longitudinal study of caries development in initially caries-free naval recruits. J Dent Res 1982:1405-1407.
37. Alaluusua S, Renkonen OV. Streptococcus mutans establishment and dental caries experience in children from 2 to 4 years old. Scand J Dent Res 1983:453-457.
38. Kingman A, Little W, Gomez I et al. Salivary levels of Streptococcus mutans and lactobacilli and dental caries experiences in a US adolescent population. Community Dent Oral Epidemiol 1988:98-103.
39. Curtiss R III. Genetic analysis of Streptococcus mutans virulence. Curr Top Microbiol Immunol 1985:253-277.
40. Kuramitsu HK. Virulence factors of mutans streptococci: role of molecular genetics. Crit Rev Oral Biol Med 1993:159-176.
41. Kuramitsu HK. Virulence properties of oral bacteria: impact of molecular biology. Curr Issues Mol Biol 2001:35-36.
42. Tanzer JM, Freedman ML, Fitzgerald RJ et al. Diminished virulence of glucan synthesis-defective mutants of Streptococcus mutans. Infect Immun 1974:197-203.
43. Li YH, Tang N, Aspiras MB et al. A quorum-sensing signaling system essential for genetic competence in Streptococcus mutans is involved in biofilm formation. J Bacteriol 2002:2699-2708.
44. Pakula R, Walczak W. On the nature of competence of transformable streptococci. J Gen Microbiol 1963:125-133.
45. Tomasz A, Hotchkiss RD. Regulation of the transformability of pneumococcal cultures by macromolecular cell products. Proc Natl Acad Sci USA 1964:480-487.
46. 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 1965:155-159.
47. Havarstein LS, Coomaraswamy G, Morrison DA. An unmodified heptadecapeptide pheromone induces competence for genetic transformation in Streptococcus pneumoniae. Proc Natl Acad Sci USA 1995:11140-11144.
48. Havarstein LS, Diep DB, Nes IF. A family of bacteriocin ABC transporters carry out proteolytic processing of their substrates concomitant with export. Mol Microbiol 1995:229-240.
49. Cheng Q, Campbell EA, Naughton AM et al. The com locus controls genetic transformation in Streptococcus pneumoniae. Mol Microbiol 1997:683-692.
50. Håvarstein LS, Gaustad P, Nes IF et al. Identification of the streptococcal competence-pheromone receptor. Mol Microbiol 1996:863-869.
51. Pestova EV, Havarstein LS, Morrison DA. Regulation of competence for genetic transformation in Streptococcus pneumoniae by an auto-induced peptide pheromone and a two-component regulatory system. Mol Microbiol 1996:853-862.
52. Claverys JP, Havarstein LS. Extracellular-peptide control of competence for genetic transformation in Streptococcus pneumoniae. Front Biosci 2002:d1798-1814.
53. Havarstein LS, Morrison DA. Quorum sensing and peptide pheromones in streptococcal competence for genetic transformation in Cell-cell signaling in Bacteria. G.M.a.W. Dunny SC, Ed, ASM Press, Washington, DC 1999:9-26.

54. Cvitkovitch DG. Genetic competence and transformation in oral streptococci. Crit Rev Oral Biol Med 2001:217-243.
55. Havarstein LS, Hakenbeck R, Gaustad P. Natural competence in the genus Streptococcus: evidence that streptococci can change pherotype by interspecies recombinational exchanges. J Bacteriol 1997:6589-6594.
56. Syvitski RT, Tian XL, Sampara K et al. Structure-Activity Analysis of Quorum-Sensing Signaling Peptides from Streptococcus mutans. J Bacteriol 2006.
57. Aspiras MB, Ellen RP, Cvitkovitch DG. ComX activity of Streptococcus mutans growing in biofilms. FEMS Microbiol Lett 2004:167-174.
58. Kreth J, Hung DC, Merritt J et al. The response regulator ComE in Streptococcus mutans functions both as a transcription activator of mutacin production and repressor of CSP biosynthesis. Microbiology 2007, In press.
59. Peterson SN, Sung CK, Cline R et al. Identification of competence pheromone responsive genes in Streptococcus pneumoniae by use of DNA microarrays. Mol Microbiol 2004:1051-1070.
60. Ween O, Gaustad P, Havarstein LS. Identification of DNA binding sites for ComE, a key regulator of natural competence in Streptococcus pneumoniae. Mol Microbiol 1999:817-827.
61. Lee MS, Morrison DA. Identification of a new regulator in Streptococcus pneumoniae linking quorum sensing to competence for genetic transformation. J Bacteriol 1999:5004-5016.
62. Luo P, Li H, Morrison DA. ComX is a unique link between multiple quorum sensing outputs and competence in Streptococcus pneumoniae. Mol Microbiol 2003:623-633.
63. Luo P, Morrison DA. Transient association of an alternative sigma factor, ComX, with RNA polymerase during the period of competence for genetic transformation in Streptococcus pneumoniae. J Bacteriol 2003:349-358.
64. Steinmoen H, Knutsen E, Havarstein LS. Induction of natural competence in Streptococcus pneumoniae triggers lysis and DNA release from a subfraction of the cell population. Proc Natl Acad Sci USA 2002:7681-7686.
65. Steinmoen H, Teigen A, Havarstein LS. Competence-induced cells of Streptococcus pneumoniae lyse competence-deficient cells of the same strain during cocultivation. J Bacteriol 2003:7176-7183.
66. Kreth J, Merritt J, Zhu L et al. Cell density- and ComE-dependent expression of a group of mutacin and mutacin-like genes in Streptococcus mutans. FEMS Microbiol Lett 2006:11-17.
67. Senadheera D, Lee AWC, Hung DC et al. Streptococcus mutans quorum-sensing system controls gtfB/C/D, ftf and gbpB expression. General meeting hosted by the American Society for microbiology. Toronto, Canada 2007.
68. Senadheera D, Mair R, Cvitkovitch DG. Streptococcus mutans competence stimulating peptide affects gtfB/C and vicR/K expression and biofilm structure. Biofilms conference hosted by the American Society for Microbiology. Quebec, Canada 2007.
69. Petersen FC, Pecharki D, Scheie AA. Biofilm mode of growth of Streptococcus intermedius favored by a competence-stimulating signaling peptide. J Bacteriol 2004:6327-6331.
70. Sablon E, Contreras B, Vandamme E. Antimicrobial peptides of lactic acid bacteria: mode of action, genetics and biosynthesis. Adv Biochem Eng Biotechnol 2000:21-60.
71. Novak J, Caufield PW, Miller EJ. Isolation and biochemical characterization of a novel lantibiotic mutacin from Streptococcus mutans. J Bacteriol 1994:4316-4320.
72. Qi F, Chen P, Caufield PW. Purification of mutacin III from group III Streptococcus mutans UA787 and genetic analyses of mutacin III biosynthesis genes. Appl Environ Microbiol 1999:3880-3887.
73. Qi F, Chen P, Caufield PW. Purification and biochemical characterization of mutacin I from the group I strain of Streptococcus mutans, CH43 and genetic analysis of mutacin I biosynthesis genes. Appl Environ Microbiol 2000:3221-3229.
74. Qi F, Chen P, Caufield PW. The group I strain of Streptococcus mutans, UA140, produces both the lantibiotic mutacin I and a nonlantibiotic bacteriocin, mutacin IV. Appl Environ Microbiol 2001:15-21.
75. Matsumoto-Nakano M, Kuramitsu HK. Role of bacteriocin immunity proteins in the antimicrobial sensitivity of Streptococcus mutans. J Bacteriol 2006:8095-8102.
76. Senadheera MD et al. A VicRK signal transduction system in Streptococcus mutans affects gtfBCD, gbpB and ftf expression, biofilm formation and genetic competence development. J Bacteriol 2005:4064-76.
77. Li YH, Lau PC, Tang N et al. Novel two-component regulatory system involved in biofilm formation and acid resistance in Streptococcus mutans. J Bacteriol 2002:6333-6342.
78. Qi F, Merritt J, Lux R et al. Inactivation of the ciaH Gene in Streptococcus mutans diminishes mutacin production and competence development, alters sucrose-dependent biofilm formation and reduces stress tolerance. Infect Immun 2004:895-899.
79. Biswas S, Biswas I. Regulation of the glucosyltransferase (gtfBC) operon by CovR in Streptococcus mutans. J Bacteriol 2006:988-998.
80. Idone V, Brendtro S, Gillespie R et al. Effect of an orphan response regulator on Streptococcus mutans sucrose-dependent adherence and cariogenesis. Infect Immun 2003:4351-4360.