A model of efficiency: Stress tolerance by *Streptococcus mutans*

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Abstract

The complete genome sequence of *Streptococcus mutans*, a bacterial pathogen commonly associated with human dental caries, was published in 2002. The streamlined genome (2.03Mb) revealed an organism that was well adapted to its obligately host-associated existence in multispecies biofilms on tooth surfaces; a dynamic environment that undergoes rapid and substantial environmental fluctuations. However, *S. mutans* lacks many of the sensing systems and alternative sigma factors that bacteria often use to coordinate gene expression in response to stress and changes in their environment. Over the past seven years, functional genomics and proteomics have enhanced our understanding of how *S. mutans* has integrated the stress regulon and global transcriptional regulators to integrate responses to environmental fluctuations with modulation of virulence in a way that ensures persistence in the oral cavity and capitalizes on conditions that are favorable for the development of dental caries. Here, we highlight advances on dissection of the stress regulon of *S. mutans* and its intimate interrelationship with pathogenesis.

Introduction

Caries is a classic biofilm disease that develops when changes in the oral environment enhance the growth of cariogenic bacteria, which are highly efficient at converting carbohydrates to the organic acids that demineralize tooth enamel. Microbiological assessment of caries-active sites and studies with experimental animals implicated *Streptococcus mutans* as the primary causative agent of human dental caries (Loesche, 1986). The virulence of *S. mutans* resides in three core attributes; its abilities to form biofilms on the tooth surface, to produce large quantities of organic acids (acidogenicity) from a wide range of carbohydrates, and to tolerate environmental stresses, particularly low pH (aciduricity) (Lemos *et al.*, 2005). In addition to dental caries, *S. mutans* is often an agent in sub-acute bacterial endocarditis, a life-threatening inflammation of heart valves.

Unlike most infectious diseases, in which classic virulence factors, such as a toxin, play a clear role in the damage elicited by the organism, the pathology of dental caries is associated

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almost exclusively with bacterial metabolism. Catabolism of the nutrients in saliva and the host's diet creates stressors in the form of acids, reactive oxygen species (ROS), and other agents that damage biomolecules. Thus, stress tolerance by the bacteria is intimately intertwined with virulence. The purpose of this review is to highlight post-genomic research on genetic, biochemical and physiologic mechanisms that have evolved in *S. mutans* to modulate its pathogenic potential in response to nutritional, chemical and physical stresses encountered in complex biofilms.

Seven Years P.G. (Post Genome)

In 2001, the complete genome sequence of a serotype *c* strain of *S. mutans* became available (Ajdic *et al.*, 2002). Through the application of functional genomics, transcriptomics and proteomics, researchers were able to make rapid progress in dissecting the mechanisms of stress tolerance utilized by this pathogen. One theme that emerged from these studies is that *S. mutans* has streamlined its genome by using pathways that cope with environmental insults to regulate a variety of virulence attributes.

A few years after the completion of the UA159 genome sequence, *S. mutans* microarray slides became available, with generous support from the NIDCR, through the J. Craig Venter Institute (formerly The Institute for Genomic Research, TIGR). To date, microarrays have been used to probe the responses of *S. mutans* to amino acid starvation (Nascimento *et al.*, 2008), oxygen (Ahn *et al.*, 2007), sugar transport (Ajdic & Pham, 2007), and manganese-depletion (Arirachakaran *et al.*, 2007); to identify genes that are differentially expressed in biofilms of *S. mutans* compared with free-living cells (Shemesh *et al.*, 2007); and to evaluate the consequences of gene-specific mutations (Abranches *et al.*, 2006; Abranches *et al.*, 2008; Lemos *et al.*, 2008; Merritt *et al.*, 2005; Nascimento *et al.*, 2008; Sztajer *et al.*, 2008; Wen *et al.*, 2006).

Proteomic studies have been instrumental in identifying proteins and pathways that participate in acid tolerance and acid adaptation (Len *et al.*, 2004a; Len *et al.*, 2004b; Rathsam *et al.*, 2005a; Rathsam *et al.*, 2005b; Welin *et al.*, 2003; Welin *et al.*, 2004; Wilkins *et al.*, 2002; Wilkins *et al.*, 2003). Of particular interest is a report by Nick Jacques and co-workers that used continuous culture to catalogue changes in the expression of proteins involved in energy metabolism when the growth pH was lowered from 7 to 5 (Len *et al.*, 2004b). By coupling proteomic data with measurements of end products of carbon utilization, the authors were able to propose that *S. mutans* tolerates growth at low pH by expending energy to extrude H^+, by modulating the production of acid end products, and by using branched chain amino acid biosynthesis as a potential mechanism to reduce acid production and moderate intracellular pH (Len *et al.*, 2004b).

Comparison of the proteome of mature biofilm and planktonic cells of *S. mutans* cells grown at neutral pH revealed that multiple proteins associated with carbon uptake and cell division were down-regulated in biofilms, whereas proteins required for the development of genetic competence were up-regulated (Rathsam *et al.*, 2005a); the latter finding being consistent with the observation that the transformation efficiency of *S. mutans* is optimal during biofilm growth (Li *et al.*, 2001b). This observation is thought to have significance in terms of

plaque ecology. Specifically, co-ordinated production of bacteriocins from *S. mutans* and the development of competence have been documented in high-cell density environments, suggesting that the organism could use competence-induced cell lysis to acquire DNA from neighboring species (Kreth *et al.*, 2005; Kreth *et al.*, 2006; Kreth *et al.*, 2007). Notably, a study with *S. mutans* implicated the presence of DNA released from competence-induced cell lysis in the extracellular matrix with proper biofilm maturation (Petersen *et al.*, 2005). It remains to be determined whether *S. mutans* biofilms acquire DNA from the external environment to provide a nutrient source, to increase genetic diversity, or both (Spoering & Gilmore, 2006). In addition to the potential impact on commensal organisms in oral biofilms, a direct correlation between production of the competence stimulating peptide (CSP) and activation of autolytic pathways with biofilm formation and persistence of *S. mutans* has been noted. In particular, when administered at doses beyond the levels necessary to induce competence, CSP of *S. mutans* was found to induce cell lysis (Qi *et al.*, 2005), suggesting the presence of an altruistic programmed cell death pathway. In this case, the "sacrifice" of a subset of cells may enable the establishment and survival of the remainder of the population.

Stress Survival Pathways

Bacteria in dental plaque experience a wide range of stresses. The intermittent ingestion of food by the host results in dramatic changes in nutrient availability and pH, but significant variability in oxygen tension and osmolality are also observed (Lemos *et al.*, 2005). Although it has been noted that oral biofilms experience a "feast or famine" lifestyle, organisms residing in the oral cavity are really not exposed to severely-oligotrophic environments. For this reason, studies of the stress responses of *S. mutans* have served as an excellent model to reveal critical differences in the ways that obligately host-associated bacteria cope with environmental stresses when compared with bacteria that have both free-living and host-associated lifestyles. These studies have revealed important contrasts in the way this organism copes with stresses and uses stress regulons/enzymes to coordinate virulence and survival compared to more widely-studied bacterial paradigms (Fig. 1).

pH homeostasis and metabolic pathways

The membrane-bound F-ATPase (H$^+$-translocating ATPase) is considered the primary determinant of acid tolerance of *S. mutans* because it allows the organism to maintain a cytoplasmic pH that is more alkaline than the extracellular environment (Lemos *et al.*, 2005). Work from the laboratory of Marquis correlates acid tolerance in oral streptococci with the pH optima and absolute level of activity of the F_1F_O-ATPase (Bender *et al.*, 1986). Moreover, it was recently demonstrated that the F-ATPase of *S. mutans*, and other oral bacteria, can function as an ATP synthase in starved cells grown at low pH (Sheng & Marquis, 2006). The authors demonstrated that in starved cells, a sudden drop in pH result in a rapid increase in ATP, followed by a rapid loss, that enhance protection against acid killing. By using specific inhibitors of F-ATPase, the authors were able to demonstrate that this increase in ATP comes from the enzyme acting as an ATP synthase. Thus, the F-ATPase may play a dual role in acid tolerance - extruding protons out of the cells and, under certain conditions, generating ATP for growth and persistence (Fig. 2).

A mechanism for acid resistance used by many oral streptococci is the production of ammonia by urease enzymes or the arginine deiminase system (ADS) (Burne & Marquis, 2000). Organisms carrying these enzymes can convert urea or arginine, respectively, to produce CO_2 and ammonia, which can neutralize acids and give the organisms a competitive advantage in acidified biofilms. Although *S. mutans* is not capable of generating significant quantities of alkali as it lacks urease and ADS pathways (Ajdic *et al.*, 2002), an agmatine deiminase system (AgDS), analogous to the ADS, was characterized in *S. mutans* UA159 (Griswold *et al.*, 2006). The AgDS converts agmatine, a decarboxylated derivative of arginine that is found in human dental plaque, to putrescine, ammonia and CO_2. Whereas the ADS and urease pathways catalyze substantial environmental alkalinization and appear to be associated with caries resistance, the AgDS of *S. mutans* is expressed at relatively low levels and is unlikely to elicit a significant alkalinization of the environment. However, the production of ammonia from agmatine is believed to contribute to the competitive fitness of *S. mutans* at low pH by increasing the cytoplasmic pH and generating ATP that can be used for growth or to extrude protons (Fig. 2) (Griswold *et al.*, 2006).

Another contributor to acid tolerance of *S. mutans* is malolactic fermentation (MLF), which catalyzes the conversion of dicarboxylic L-malate, a major acid in fruits such as apple, to the monocarboxylic lactic acid and CO_2. It was demonstrated that although malate did not serve as a catabolite for growth of *S. mutans*, it did serve to protect the organism against acid killing by increasing the pH of the cytoplasm via production of CO_2 (Fig. 2) (Sheng & Marquis, 2007).

Protection, Repair and Quality Control of Macromolecules—One consequence of exposure to environmental stresses is the accumulation of abnormal proteins due to increased errors in transcription and translation. Moreover, aging cells present in mature biofilms are prone to mistranslation and aggregation. In this context, molecular chaperones and proteases, which modulate the stability of proteins and prevent accumulation of misfolded proteins, are central to physiologic homeostasis. In support to this concept, proteome analysis of *S. mutans* grown at steady-state in continuous culture at pH 7 or 5 identified several molecular chaperones, proteases and DNA repair enzymes as up-regulated during growth at low pH (Len *et al.*, 2004a).

The GroEL and DnaK chaperones take part in several cellular processes, including protein folding, renaturation, and presentation of proteins for degradation. In *S. mutans*, DnaK and GroEL appear to be indispensable, and the essential nature of these chaperones was confirmed by forced down-regulation of *groEL* and *dnaK* expression (Lemos *et al.*, 2007b). Lowering of DnaK levels resulted in impaired capacity to form biofilms in the presence of glucose and rendered the strain more sensitive to low pH, elevated temperature and H_2O_2 (Lemos *et al.*, 2007b). The acid-sensitivity of the DnaK knock-down strain was attributed, at least in part, to the DnaK chaperone participating in the biogenesis or stabilization of the F-ATPase complex (Lemos *et al.*, 2007b). Downregulation of GroEL also resulted in high temperature sensitivity and impaired capacity to form biofilms, but did not affect growth at low pH or in the presence of H_2O_2(Lemos *et al.*, 2007b). Wen and colleagues showed that the ribosome-associated peptidyl-prolyl isomerase RopA (trigger factor) is important for adherence and formation of biofilms and for tolerance to low pH and H_2O_2 (Wen *et al.*,

2005). Inactivation of the surface-associated HtrA protease or the cytoplasmic ClpP peptidase generated multiple stress-sensitive phenotypes in *S. mutans*, and was also linked to altered biofilm formation and reduced genetic competence (Ahn *et al.*, 2005; Biswas & Biswas, 2005; Deng *et al.*, 2007b; Lemos & Burne, 2002). Notably, in all these cases, a unifying theme is the intimate connection between stress responses and biofilm formation, suggesting that the stress regulon of *S. mutans* may be responsible for controlling a broader set of biological functions when compared to organisms with more complex genomes.

Many of the stresses encountered by oral bacteria induce DNA damage, in particular acid and oxidative stresses increase the formation of abasic sites in DNA. Earlier reports have demonstrated an overlap between DNA repair systems and stress response pathways, including RecA, the endonuclease Smn and the UV repair excinuclease UvrA (Hahn *et al.*, 1999; Hanna *et al.*, 2001; Quivey *et al.*, 1995). More recently, Faustoferri and colleagues characterized the Smx exonuclease in *S. mutans* and showed that an *smx* mutant strain was highly sensitive to DNA damage caused by the production of hydoxyl radicals via Fenton reaction (Faustoferri *et al.*, 2005).

Cell Envelope Alterations—The importance of cell membrane integrity and composition in relation to changes that affect proton permeability and F-ATPase activity in *S. mutans* has been documented (Lemos *et al.*, 2005). Fozo and Quivey showed that, in response to the acidification of its environment, *S. mutans* increases the proportion of monounsaturated membrane fatty acids (Fozo & Quivey, 2004b), which is thereby predicted to decrease proton permeability. Inactivation of the gene responsible for biosynthesis of monounsaturated fatty acids, *fabM*, resulted in a strain that was extremely sensitive to low pH and unable to maintain pH (Fozo & Quivey, 2004a). Rats infected with the *fabM* mutant exhibited substantially reduced caries, as compared to the parent strain (Fozo *et al.*, 2007).

The significance of membrane protein biogenesis to stress tolerance was demonstrated in a study with mutated strains lacking the signal recognition particle-translocation (SRP) pathway or the membrane-localized chaperone YidC, both involved in the translocation and assembly of membrane proteins. Once considered essential for the viability of all organisms, the SRP pathway was found to be dispensable in *S. mutans* (Hasona *et al.*, 2005), although mutants lacking proteins of the SRP pathway or YidC were impaired in growth under a variety of stress conditions (Hasona *et al.*, 2005). The authors observed that YidC and a functional SRP pathway is necessary for optimal insertion of membrane proteins, including the F-ATPase, providing a partial explanation for the diminished acid tolerance of strains lacking YidC or components of the SRP pathway (Hasona *et al.*, 2005; Hasona *et al.*, 2007). Notably, mutations in SRP-related genes were also associated with decreases in biofilm formation, providing another example of the overlap between pathways that govern stress tolerance and biofilm formation (Hasona *et al.*, 2007).

Finally, the surface-associated protein BrpA was found to play a role in biofilm development, autolysis, cell division and stress tolerance (Wen *et al.*, 2006). A comparison of the transcriptomes of the *brpA* mutant and the parent reveal significant alterations in the expression of genes involved in cell wall biogenesis, stress tolerance and adherence (Wen *et*

al., 2006). Although the function of BrpA has not been defined, increased autolysis in the *brpA* strain indicates that this protein may play a role in modulating cell wall integrity through modulation of autolytic activities, which could mechanistically link BrpA to the increases in susceptibility to acid and oxidative stresses observed in the BrpA-deficient strain (Wen *et al.*, 2006).

Two-component signal transduction systems—*S. mutans* lives almost exclusively in densely-populated biofilms that form on the tooth surface. The structure and composition of these biofilms are influenced by the capacity of its constituents to adapt to environmental changes. As is typical of bacteria with specialized niches, there are very few alternative sigma factors in the UA159 genome (Ajdic *et al.*, 2002). Thus, regulatory systems, such as two-component systems (TCS), which integrate various chemical and physical signals to coordinate appropriate gene expression patterns, play a central role in stress tolerance and are viewed as desirable targets for the development of new antimicrobial therapies.

TCS are composed of a transmembrane sensor kinase that detects environmental changes and a cytosolic response regulator, which is a DNA binding protein that modulates expression of target genes when phosphorylated by the kinase. In streptococcal species, the number of TCS is small compared to organisms with a free living life-style, ranging from as few as 10 in *S. thermophilus*, to more than 20 in *S. agalactiae*. Sequence analysis initially revealed the presence of 13 TCS in *S. mutans* UA159 (Ajdic *et al.*, 2002), but the Biswas lab identified an additional pair in this same strain (Biswas *et al.*, 2008).

Over the past few years, studies that evaluated the role of TCS in *S. mutans* have shown that they regulate virulence gene expression, induction of competence, biofilm development, bacteriocin production and stress tolerance (Biswas *et al.*, 2008; Chen *et al.*, 2007; Deng *et al.*, 2007a; Levesque *et al.*, 2007; Li *et al.*, 2001a; Li *et al.*, 2002a; Li *et al.*, 2002b; Qi *et al.*, 2004; Senadheera *et al.*, 2005; Zeng *et al.*, 2006). In particular, two studies from independent laboratories systematically inactivated the genes encoding sensor kinases of all TCS and evaluated their role in stress tolerance by *S. mutans* (Biswas *et al.*, 2008; Levesque *et al.*, 2007). In the study by Lévesque and colleagues, *smu1814c* (*scnK)* and *smu1965c* (*levS*) mutants displayed significantly slower growth at pH 5.5, whereas the *smu1128c* (*ciaH*) mutant grew better than the parental strain in the presence of NaCl or H_2O_2 (Levesque *et al.*, 2007). Biswas and co-workers found that inactivation of three sensor kinases, *smu486* (*liaS*), *smu1128c* (*ciaH*) and *smu1516c* (*vicK*), affected stress tolerance of strain UA159 (Biswas *et al.*, 2008). However, the *vicK* mutant showed an increased tolerance to puromycin, which causes premature chain termination during protein synthesis (Biswas *et al.*, 2008). The *liaS* and *ciaH* mutants showed reduced growth when incubated in aerobic conditions or on agar medium supplemented with H_2O_2 (Biswas *et al.*, 2008). The *liaS* and *ciaH* mutants also showed increased sensitivity to puromycin, while the *ciaH* mutant showed significant reduction of growth at pH 5 and displayed increased sensitivity to DNA damage caused by mitomycin C (Biswas *et al.*, 2008). Notably, previous reports have also shown that inactivation of *ciaH* resulted in an acid sensitive phenotype in strains UA159 and UA140 (Ahn *et al.*, 2006; Qi *et al.*, 2004). The *S. mutans* VicRK system was shown to respond to, and protect against, oxidative stress in one particular study (Deng *et al.*, 2007a). A role in oxidative stress response was also assigned to ScnRK, as *scnRK* mutants were more

sensitive to H_2O_2 and more susceptible to phagocytic killing in non-activated macrophages (Chen *et al.*, 2007). Studies from the Cvitkovitch lab have shown that inactivation of LiaS or ComDE conferred an acid-sensitive phenotype to strains NG8 and BM71, respectively (Li *et al.*, 2001a; Li *et al.*, 2002a), although *comD* (*smu1916c*) or *comE* (*smu1917c*) do not appear to affect acid tolerance in strain UA159 (Ahn *et al.*, 2006). Finally, the *smu927-smu928* TCS, designated *relRS*, that is co-transcribed with a newly described (p)ppGpp-synthetase (*relP*) has been implicated in survival and persistence as it may help regulate (p)ppGpp metabolism (Lemos *et al.*, 2007a).

In *S. pyogenes*, the TCS CovRS regulates expression of approximately 15% of the genome, including key virulence genes (Graham *et al.*, 2002). In *S. mutans* UA159, CovR is an orphan response regulator that controls expression of genes related to biofilm formation and virulence (Biswas *et al.*, 2007; Biswas & Biswas, 2006). Similar to what has been observed for the *S. pyogenes covRS*, expression of the *S. mutans covR* is autoregulated, optimal during exponential-growth and induced by addition of Mg^{2+} in a dose-dependent manner (Chong *et al.*, 2008). The extent of the genes controlled by CovR in *S. mutans* is not known but based on the findings obtained in other streptococci, it is expected that CovR participate in the stress responses.

Collectively, these data support the idea that there may be substantial heterogeneity among strains in the role of specific TCS, not only in the genes they regulate, but also in the external stimuli to which they respond. Nevertherless, CiaRH have been consistently found to play a role in the stress responses by *S. mutans*. Moreover, CiaRH have also been implicated in competence development, bacteriocin production and biofilm formation (Ahn *et al.*, 2006; Levesque *et al.*, 2007; Qi *et al.*, 2004). More recently, it was demonstrated that the *ciaRH* operon of *S. mutans* consists of three genes with the first gene, *ciaX*, encoding a small, double-glycine signaling peptide that allows CiaRH to modulate its own expression in response to calcium (He *et al.*, 2008). Inactivation of *ciaX*, or point mutations in its calcium-binding domain resulted in diminished biofilm formation that was rescued by addition of calcium. Human saliva is saturated in calcium (Agha-Hosseini *et al.*, 2006) and calcium is the principal cation in tooth enamel, so calcium signaling may be an important regulator, through CiaRH, of stress responses and virulence in *S. mutans*.

Other regulators—Metal ions, including iron and manganese, have been implicated in the regulation of virulence expression by *S. mutans*. In particular, the SloR metalloregulator was shown to modulate *S. mutans* biofilm formation, genetic competence and oxidative stress tolerance in response to manganese availability (Rolerson *et al.*, 2006). Work from the Spatafora lab has linked SloR repression of the transcriptional regulator *gcrR* with acid stress tolerance (Dunning *et al.*, 2008). More specifically, a *gcrR* mutant was more sensitive to low pH and this phenotype was linked to the inability of the mutant to maintain pH homeostasis.

As mentioned above, the AgDS has been proposed to enhance acid resistance through alkalinization of the cytoplasm (Griswold *et al.*, 2006). The AgDS of *S. mutans* is subject to complex regulation by substrate, catabolite control, and relevant environmental stresses. A LuxR-like transcriptional regulator, named *aguR*, was identified upstream the *aguBDAC*

operon. Inactivation of *aguR* decreased AgD activity and eliminated agmatine induction, indicating that AguR is a major regulator of AgDS (Griswold *et al.*, 2006).

Nutritional Regulators and alteration of catabolic pathways—In order to thrive in dental plaque where there is considerable fluctuation in the nutrient pools, *S. mutans* must be able to adjust its metabolism and gene expression patterns to maximize the use of available substrates (Lemos *et al.*, 2005). Despite the need to endure periods of nutrient limitation, abrupt exposure to an excess amount of carbohydrate in the diet can result in the rapid accumulation of toxic glycolytic intermediates, acidification of the environment, and osmotic stress. To survive nutrient starvation, to cope with the detrimental effects of glycolytic intermediates, and to maintain proper NAD/NADH$^+$ balances, *S. mutans* has developed a sophisticated regulatory nextwork that combines transcriptional regulation with allosteric modulation of enzymatic activities to coordinate optimal flow of carbohydrates.

Carbohydrate source and availability are key factors affecting the pathogenic potential of oral biofilms. The sugar phosphotransferase system (PTS) is the major carbohydrate transport system in oral streptococci, especially under carbohydrate-limiting conditions. In addition to participating in sugar uptake, PTS components influence many other cellular processes. Mutations in the ManL PTS permease influenced biofilm development, regulation of acid tolerance and global control of gene expression, in particular carbon catabolite repression (Abranches *et al.*, 2006; Abranches *et al.*, 2008). Two global regulators of central metabolism genes, CcpA and CodY, have been shown to impact acid tolerance and the expression of other virulence traits of *S. mutans* (Abranches *et al.*, 2008; Lemos *et al.*, 2008). CcpA, a regulator of carbon-metabolism in Gram-positive bacteria, has been shown to globally regulate transcription in response to carbohydrate availability, and a CcpA-deficient strain was substantially more acid resistant than its parent (Abranches *et al.*, 2008). The enhanced acid tolerance of the CcpA mutant has been associated with increases in the expression of the PTS that result in higher rates of ATP generation through glycolysis. Microarrays revealed that CodY, a regulator that helps cells to adapt to poor nutritional conditions, is indeed a global regulator of gene expression in *S. mutans* (Lemos *et al.*, 2008). Phenotypic studies revealed that the *codY* mutant had reduced capacities to form biofilms and was more sensitive to growth at low pH (Lemos *et al.*, 2008).

The nutritional alarmone (p)ppGpp also appears to play an important role in orchestrating an appropriate response to multiple environmental and physiologic inputs that *S. mutans* encounters in the oral cavity (Fig. 3). When limited for essential amino acids, bacteria accumulate (p)ppGpp by enzymatic phosphorylation of GDP and GTP, resulting in down-regulation of genes for macromolecular biosynthesis and up-regulation of genes for amino acid biosynthesis and stress tolerance. In Gram-positive bacteria (GPB), RelA is a bi-functional enzyme with potent (p)ppGpp-synthetic and -degradative activities. In *S. mutans*, RelA was shown to play major roles in the regulation of phenotypic traits that are required for establishment, persistence and survival (Lemos *et al.*, 2004; Nascimento *et al.*, 2008), further supporting an overlap between circuits that govern nutrient starvation, general stress tolerance and biofilm fomation. Until recently, RelA was considered the sole enzyme responsible for synthesis and degradation of (p)ppGpp in Gram-positive bacteria. However, our group recently identified two novel enzymes, designated RelP and RelQ, with (p)ppGpp-

synthase activities in *S. mutans* that could be found in a number of related GPB (Lemos *et al.*, 2007a). A *relAPQ* triple mutant was auxotrophic for the branched-chain amino acids (BCAA) leucine and valine, but not isoleucine, a phenotype that was directly related to CodY-dependent repression of genes involved in the synthesis of BCAA (Lemos *et al.*, 2008). As mentioned above, RelP is co-transcribed with, and apparently regulated by, the RelRS TCS (Lemos *et al.*, 2007a) suggesting that *S. mutans* may use environmental signals to optimize cell growth and survival in a manner that allows the organism to balance growth during dietary intake by the host with the capacity to rapidly mount an adaptive response during fasting periods. Consistent with the role of (p)ppGpp in bacteria, homologues of RelRS in *S. pyogenes*, designated SptRS, were shown to be critical for this bacterium to survive in saliva (Shelburne *et al.*, 2005).

Concluding Remarks

Genomic and proteomic studies have enabled researchers to make rapid progress on the identification of genes, proteins and pathways that are associated with stress tolerance in *S. mutans*. Because there is a strong overlap between stress tolerance and biofilm development pathways, some of these gene products are attractive targets for the development of new anti-caries therapies (Matsushita & Janda, 2002). In particular, strategies that short-circuit regulatory pathways used by *S. mutans* to sense and respond to environmental signals may have a potent capacity to disrupt cariogenic biofilms.

Acknowledgments

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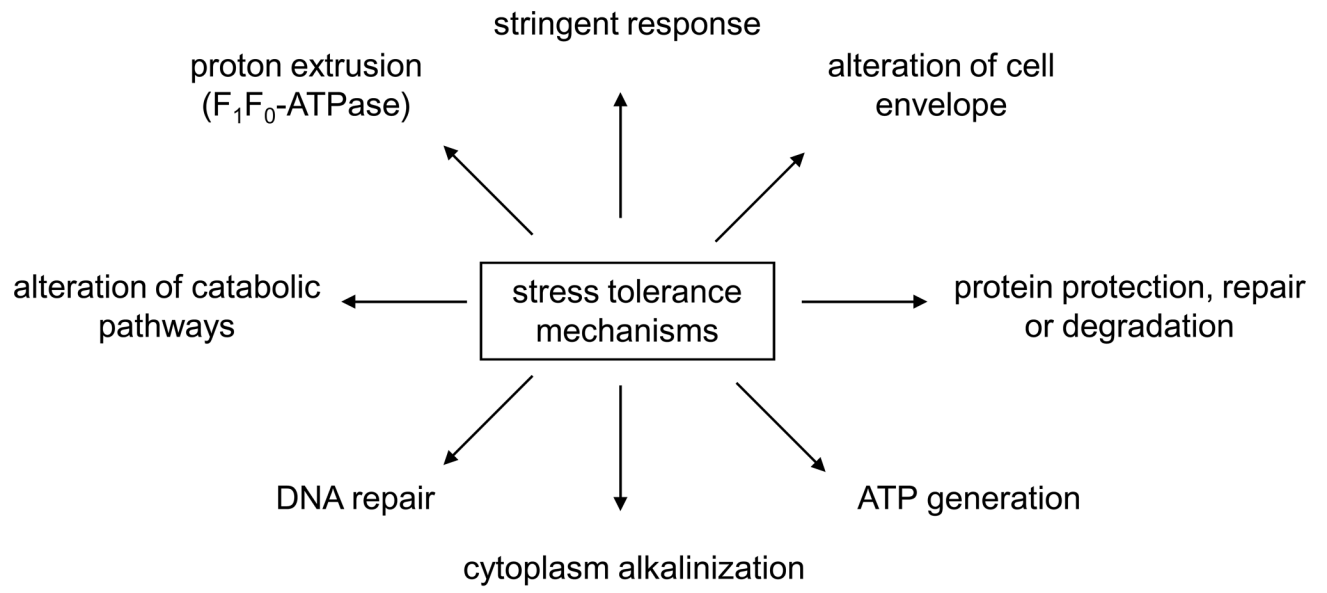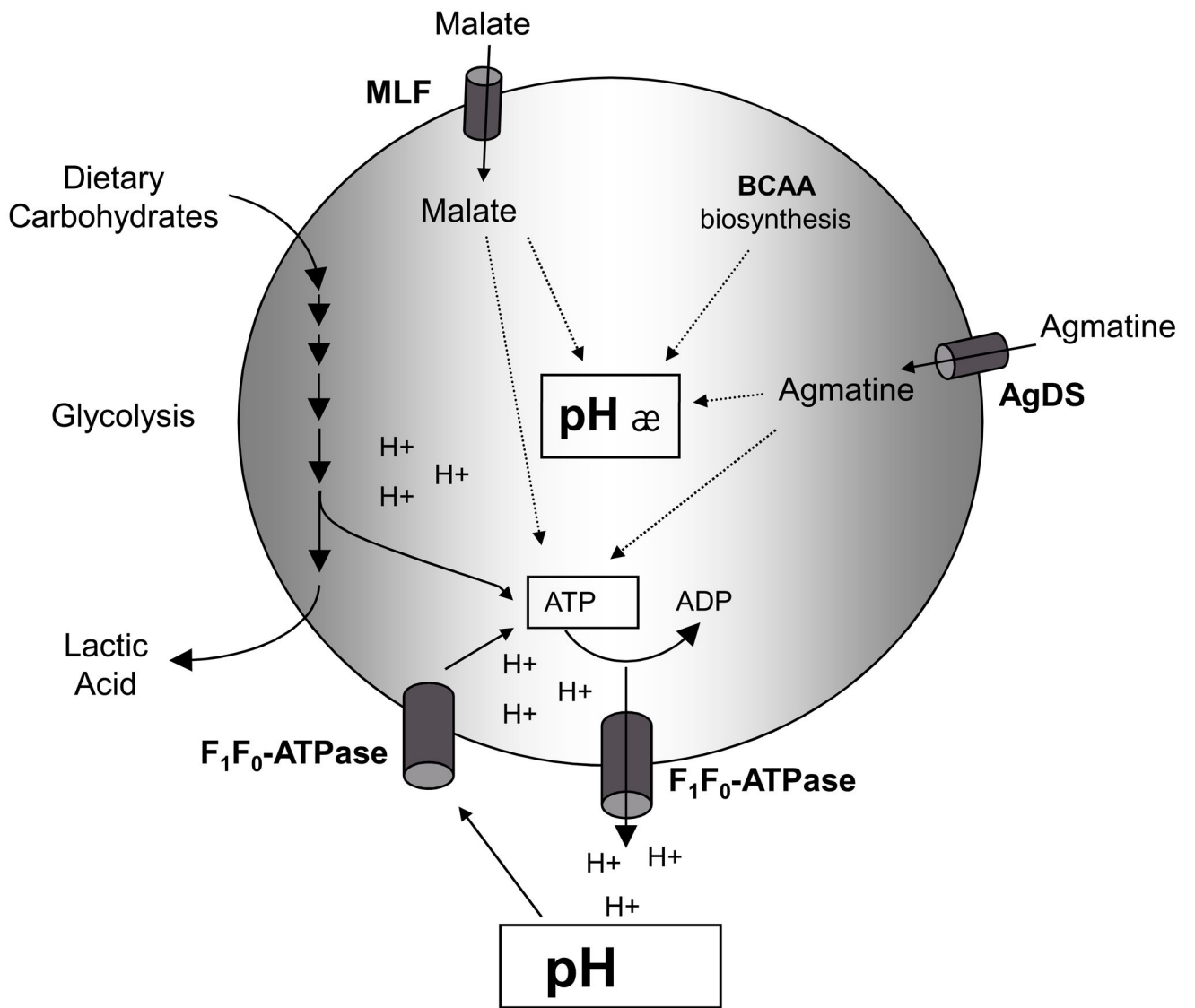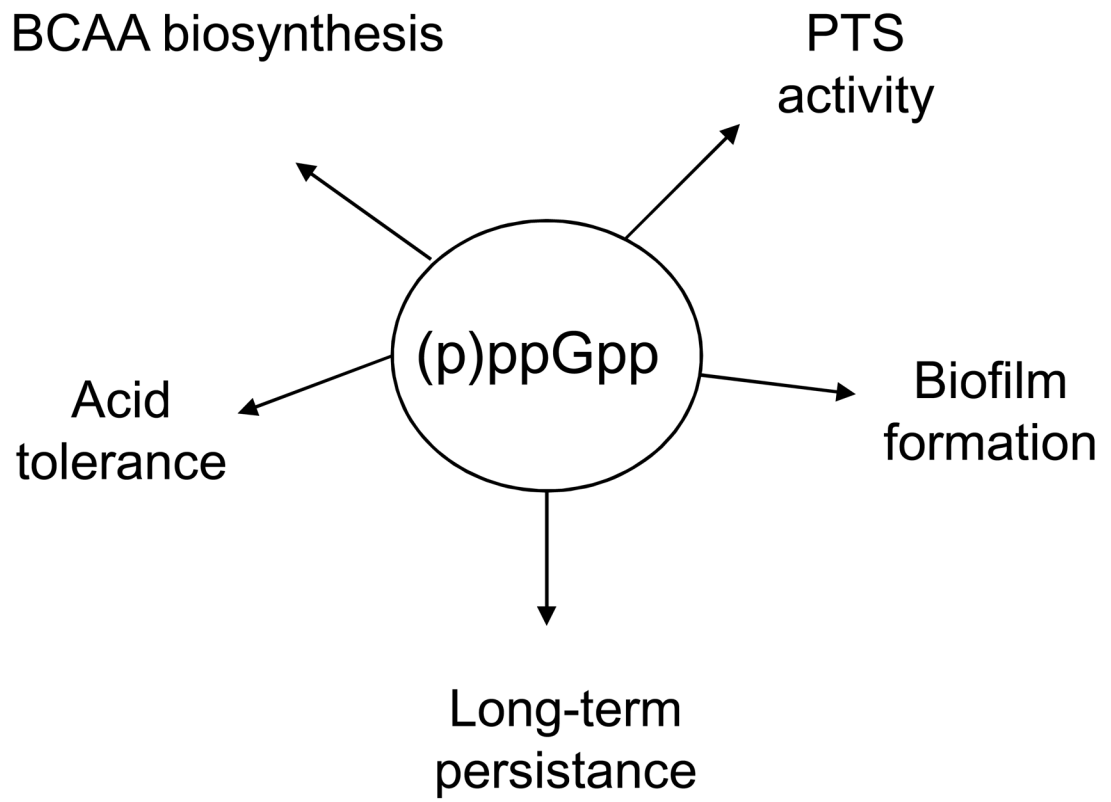
Figure 1.

Figure 2.

BCAA biosynthesis   PTS activity

Acid tolerance   (p)ppGpp   Biofilm formation

Long-term persistance

Figure 3.