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The Biology of *Streptococcus mutans*

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ABSTRACT As a major etiological agent of human dental caries, *Streptococcus mutans* resides primarily in biofilms that form on the tooth surfaces, also known as dental plaque. In addition to caries, *S. mutans* is responsible for cases of infective endocarditis with a subset of strains being indirectly implicated with the onset of additional extraoral pathologies. During the past 4 decades, functional studies of *S. mutans* have focused on understanding the molecular mechanisms the organism employs to form robust biofilms on tooth surfaces, to rapidly metabolize a wide variety of carbohydrates obtained from the host diet, and to survive numerous (and frequent) environmental challenges encountered in oral biofilms. In these areas of research, *S. mutans* has served as a model organism for ground-breaking new discoveries that have, at times, challenged long-standing dogmas based on bacterial paradigms such as *Escherichia coli* and *Bacillus subtilis*. In addition to sections dedicated to carbohydrate metabolism, biofilm formation, and stress responses, this article discusses newer developments in *S. mutans* biology research, namely, how *S. mutans* interspecies and cross-kingdom interactions dictate the development and pathogenic potential of oral biofilms and how next-generation sequencing technologies have led to a much better understanding of the physiology and diversity of *S. mutans* as a species.

In 1924, J. Clarke isolated an organism from carious lesions and called it *Streptococcus mutans*, because he thought the oval-shaped cells observed were mutant forms of streptococci (1). However, it was in the late 1950s when *S. mutans* gained widespread attention within the scientific community, and by the mid-1960s, clinical and animal-based laboratory studies depicted *S. mutans* as an important etiologic agent in dental caries (2). The natural habitat of *S. mutans* is the human oral cavity, more specifically, the dental plaque, a multispecies biofilm formed on hard surfaces of the tooth. It has been largely accepted that the cariogenic potential of *S. mutans* resides in three core attributes: (i) the ability to synthesize large quantities of extracellular polymers of glucan from sucrose that aid in the permanent colonization of hard surfaces and in the development of the extracellular polymeric matrix *in situ*, (ii) the ability to transport and metabolize a wide range of carbohydrates into organic acids (acidogenicity), and (iii) the ability to thrive under environmental stress conditions, particularly low pH (aciduricity) (3). While *S. mutans* does not act alone in the development of dental caries, studies from several laboratories have convincingly demon-

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strated that *S. mutans* can alter the local environment by forming an extracellular polysaccharide (EPS)-rich and low-pH milieu, thereby creating a favorable niche for other acidogenic and aciduric species to thrive. As a human pathogen, *S. mutans* is also implicated in subacute bacterial endocarditis, a life-threatening inflammation of heart valves, while a subset of strains has been linked to other extraoral pathologies such as cerebral microbleeds, IgA nephropathy, and atherosclerosis.

Strains of *S. mutans* can be classified into four serological groups (*c*, *e*, *f*, and *k*) based on the composition of cell-surface rhamnose-glucose polysaccharide, with ~75% of strains isolated from dental plaque belonging to serotype *c*, ~20% to serotype *e*, and the remaining 5% classified as serotypes *f* or *k* (4). While biochemical and genetic approaches to dissect the biology of *S. mutans* have been used for at least the past 4 decades, the publication of the complete genome sequence of the *S. mutans* strain UA159 in 2001 (5) dramatically changed the landscape, and today, *S. mutans* is one of the best-characterized Gram-positive pathogens. In this article, we highlight some of the key studies that have led to our current understanding of *S. mutans* genetics, physiology, and virulence. For a historical perspective and complete survey of the field, we direct the reader to the review articles in references 2, 3, and 6–9.

GENETIC AND PHENOTYPIC HETEROGENEITY

The first *S. mutans* genome sequenced (serotype *c* strain UA159) was found to contain ~2.0 Mb of DNA and to encode approximately 2,000 genes (5). As the cost of next-generation sequencing technologies has gone down, genomes from dozens of *S. mutans* strains have been sequenced and assembled and are now available on public databases such as the NCBI (www.ncbi.nlm.nih.gov/assembly/?term=streptococcus+mutans). This influx of genome sequences has led to an increase in comparative genomic studies focused on *S. mutans* (10–13). One of the first such studies was based on the shotgun genome sequence from 57 geographically diverse *S. mutans* clinical isolates. This study concluded that the *S. mutans* pan-genome contains a minimum of ~3,300 possible genes and has a core genome (genes that are common to all strains) of 1,490 genes (14). This means that in any one *S. mutans* isolate, ~500 genes could be distinct from any other strain, perhaps significantly influencing virulence potential or fitness. The same group, using population demographic analysis based on single nucleotide polymorphisms of the core genes, determined that a large expansion took place in the *S. mutans* population between 3,000 and 10,000 years ago, which coincided with the advent of human agriculture and increased consumption of carbohydrates in the human host diet (14). This study also identified 73 unique core genes that are found only in *S. mutans* and not in its closest relatives, many of which are involved in carbohydrate metabolism and acid resistance (14). In a follow-up study, Palmer et al. (15) characterized 15 of the most genetically diverse isolates of the 57 strains sequenced by Cornejo et al. (14) and found great variation in the phenotypes directly related to virulence, including the ability to form biofilm in the presence of sucrose and the ability to tolerate low pH and oxidative stresses, suggesting that not all strains of *S. mutans* are equally virulent and providing a rational explanation for why attempts to correlate the carriage of certain genotypes of *S. mutans* with the incidence of dental caries has proven so difficult (10, 16–18).

Work to characterize the unique core and noncore genes is ongoing and will likely lead to a greater understanding of the physiology and diversity of *S. mutans* as a species. For example, one of the unique core hypothetical genes, SMu.1147, which encodes a small peptide, was found to regulate genetic competence and other traits of general importance to *S. mutans* virulence (19). In contrast, efforts to characterize the noncore genes indicate that they likely provide a competitive advantage under particular circumstances. Such is the case for a galactose-specific phosphotransferase system (PTS) transporter found in several strains (20). Among the many noncore genes of *S. mutans* identified in recent years, those encoding the collagen-binding proteins (CBPs) Cnm and Cbm were shown to confer adhesion to collagen and laminin, invasion of endothelial and epithelial cells, and virulence in the *Galleria mellonella* invertebrate model (21, 22) as well as in rabbit and rat models of infective endocarditis (23, 24). Epidemiological studies indicate that *cnm* is present in approximately 15% of *S. mutans* isolates, whereas *cbm* is rarely found (~2%) (21, 22). Genes encoding CBPs have an uneven distribution among the different serotypes and are found at higher frequency among serotype *e*, *f*, and *k* strains but are rarely present in serotype *c* strains (25). Notably, CBP^+ *S. mutans* strains are more frequently isolated from dental plaque of individuals with bacteremia and infective endocarditis, suggesting a correlation between the production of these adhesins and systemic infections (26). Interestingly, the presence of *cnm* in *S. mutans* strains isolated from saliva has been linked to IgA nephropathy, cerebral microbleeds, and cognitive impair-

ment (27, 28). Finally, animal and clinical studies have suggested an association of CBPs in caries risk and severity (27, 29, 30).

CARBOHYDRATE METABOLISM

As a lactic acid bacterium, *S. mutans* relies exclusively on glycolysis for energy production (Fig. 1). A signature characteristic of this organism is its ability to metabolize a large variety of carbohydrates. The genome of the UA159 type strain encodes 14 phosphoenolpyruvate-dependent sugar:PTSs with specificities for various mono- and disaccharides, as well as two ATP-binding cassette (ABC) transporters involved primarily in internalizing oligosaccharides (5). Sucrose is a β2,1-linked disaccharide composed of glucose and fructose that has proven, for a number of reasons, to be the most cariogenic of all carbohydrates. *S. mutans* has evolved multiple pathways to catabolize sucrose for acid production (31), and several glycosyltransferase enzymes (Gtfs) convert sucrose into a glue-like extracellular polymer glucan that promotes the buildup of biofilms by way of cellular attachment to dental surfaces and other oral microorganisms (7). As discussed in more detail below, recent multispecies modeling studies have confirmed the unique roles of Gtfs in the formation of a heterogeneous, diffusion-limiting, low-pH matrix that is conducive to both dental demineralization and the eventual dominance by acid-tolerant species (7, 32).

Carbon Catabolite Repression

Most bacteria have evolved regulatory capacities that enable them to respond efficiently to changes in carbon source and thereby shift gene regulation, a phenomenon called carbon catabolite repression (33, 34). The majority of low-GC Gram-positive bacteria depend on a LacI-type transcriptional regulator called CcpA to ensure that catabolic genes for less preferred carbohydrates are suppressed when a preferred carbohydrate (normally glucose) is present. Interestingly, *S. mutans* deviates from this paradigm in that CcpA plays a less direct role. Instead, the glucose/mannose-PTS (EII^{Man}) system regulates the expression of catabolic operons required for assimilating secondary carbohydrates such as oligo- and disaccharides (35). Inactivation of *manL*, which encodes the A and B domains of the EII^{Man} permease, results in enhanced expression of the *fruAB* and *levDEFG* (EII^{Lev}) operons. These operons contain genes which encode two different fructanases, FruAB for hydrolyzing fructose polymers (36), and a fructose/mannose-PTS (37), respectively. As a result, a *manL* mutant demonstrates improved growth on media supplemented with fructose polymers such as inulin or levan (35). CcpA and its cofactor HPr-Ser-P are also involved in this regulation (38, 39). Whereas CcpA interacts directly with cognate *cis*-elements called *cre* (catabolite response element) near the *fruA* promoter, ManL influences *fruAB* and *levDEFG* expression independently of CcpA (39). It is now believed that EII^{Man}-dependent glucose transport influences carbohydrate metabolism via substrate-specific signaling, which occurs primarily at submillimolar concentrations of carbohydrate (38). However, substrate-level regulation is also influenced indirectly by CcpA under excess-carbohydrate conditions, since CcpA appears to regulate the transcription of *manL* (38, 39). A transcriptomic study of a *manL* deletion strain validated the key roles of EII^{Man} in global gene regulation (40), and transcriptional studies of a *ccpA* deletion strain revealed both a central role for CcpA in controlling carbon flux and the existence of a substantial network of CcpA-independent genes (41, 42).

Catch and Release

To further explore substrate-dependent carbon catabolite repression in *S. mutans*, Zeng et al. assessed the relative contributions of various sucrolytic enzymes (31). The majority (>95%) of sucrose encountered by *S. mutans* is believed to be internalized via the EII^{Scr} PTS (43, 44), while the rest is likely metabolized extracellularly by the glucosyltransferases (GTFs) and fructosyltransferase. Enhanced transcription of the *fruAB* and EII^{Lev} operons was observed in the presence of sucrose, with such activation requiring both the sucrose PTS (ScrA) and the LevQRST regulatory pathways. LevQRST is an unconventional four-component system that activates transcription of *fruAB* and EII^{Lev} in response to extracellular fructose or mannose (37, 45). Since free fructose was detected in the culture media of an *S. mutans* strain deficient in all extracellular sucrolytic enzymes after treatment with sucrose (46), it was hypothesized that following sucrose internalization and subsequent cleavage of sucrose-6-P into glucose-6-P and unphosphorylated fructose by the ScrB hydrolase, some of the fructose is released into the medium and can activate the LevQRST circuit but is insufficient to trigger CcpA-dependent carbon catabolite repression. Such a strategy of carbohydrate metabolism would be energetically efficient and avoid perturbing intracellular ATP levels, which is particularly important to a bacterium such as *S. mutans* that actively pumps protons out of the cell at the expense of ATP to maintain pH homeostasis. Interestingly, fructose may not be the only carbohydrate to be

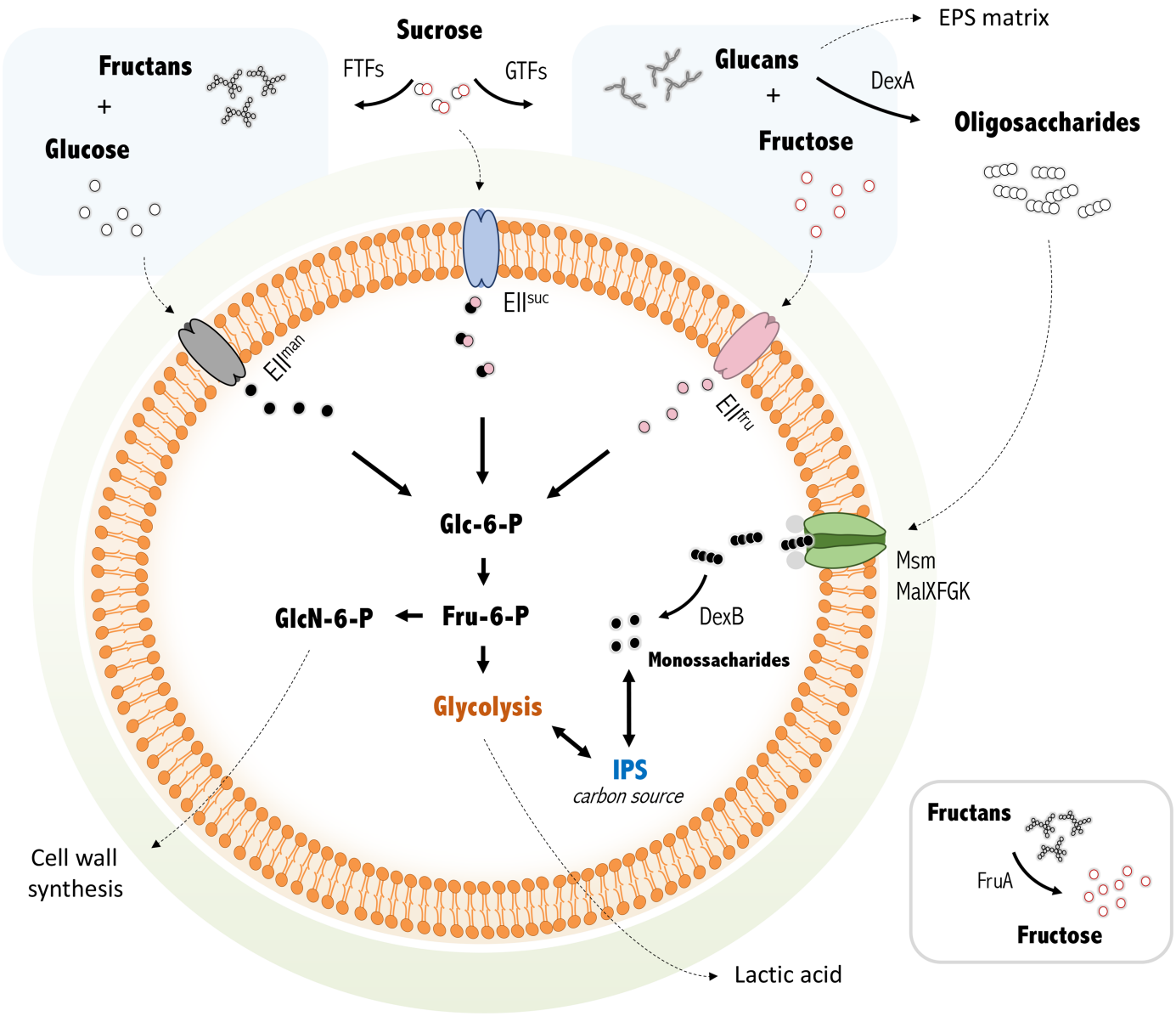

FIGURE 1 Carbohydrate metabolism in *S. mutans*. While *S. mutans* can metabolize a large variety of carbohydrates, the figure shows the metabolism of most common dietary sugars (fructose, glucose, and sucrose). Sucrose is a β2,1-linked disaccharide composed of glucose and fructose that has proven to be the most cariogenic of all carbohydrates. In the extracellular environment, sucrose is a substrate of glucosyltransferase (GTF) and fructosyltransferase (FTF) enzymes, which catalyze the production of glucans and fructans, respectively. The formation of glucans plays a key role in virulence, because they contribute to biofilm buildup by forming a glue-like polysaccharide matrix. Fructans serve as short-term extracellular carbohydrate sources and are degraded by the fructanase enzyme FruA, yielding fructose, which can be internalized for energy production. Glucans are susceptible to the action of an extracellular dextranase, DexA, which breaks down the α1,6-linkages, thereby yielding oligosaccharides (e.g., maltodextrans). After being transported into the cell, oligosaccharides are degraded into monosaccharides by the action of the DexB glucosidase. Oligosaccharides are primarily transported into the cells by ATP-binding cassette (ABC) transporters (e.g., Msm and MalXFGK transport systems), whereas monosaccharides (e.g., glucose and fructose) and disaccharides (e.g., sucrose) are predominantly taken up by the phosphoenolpyruvate:sugar PTS. In *S. mutans*, multiple PTSs can transport the same carbohydrate, with at least three PTSs being involved in fructose transport and several PTSs and permeases being involved in glucose transport. In the intracellular environment, carbohydrates are phosphorylated and processed to fructose-6-phosphate (Fru-6-P) and fermented by glycolysis with production of organic acids, mainly lactic acid. In addition, glucosamine-6-phosphate (GlcN-6-P) is synthesized from Fru-6-P and serves as an initial precursor for cell wall biosynthesis. Cells can synthesize an intracellular polysaccharide (IPS), a polymer of the glycogen-amylopectin type, when carbohydrates are in excess that can be stored as intracellular granules and are used as an energy source reserve during starvation.

released and reinternalized by *S. mutans*. Recent studies have characterized maltose and maltooligosaccharide metabolism and demonstrated the presence of free glucose in the culture medium that is apparently reinternalized by way of the glucose-PTS EII^{Man} (47, 48). Transient expulsion of glucose was also observed when *S. mutans* was grown on a mixture of glucose and lactose (49). Considering diffusion limitations within biofilm matrices (50), hexose release may likely serve as temporary energy storage and/or as a carbohydrate source for neighboring cells that may not be actively invested in metabolizing these disaccharides. This would thereby represent a bet-hedging strategy within the population and also contribute to a mutualistic relationship with other microorganisms that are closely associated with *S. mutans* (51, 52).

BIOFILM FORMATION

As a major etiological agent of human dental caries, *S. mutans* lives primarily in biofilms on the tooth surfaces, the so-called dental plaque. Strains of *S. mutans* produce up to three GTFs, GtfB, -C and, -D, that utilize the glucose moiety of sucrose as the substrate to synthesize glucose polymers of glucans (also known as mutans) (Fig. 1) (7). GtfB synthesizes water-insoluble glucans rich in α(1-3)-linkages, GtfC produces a mixture of soluble glucans rich in α(1-6)-linkages and insoluble glucans, and GtfD makes primarily soluble glucans (often called dextran). These polymers, especially the α3,1-linked water-insoluble glucans, are major constituents of plaque biofilm matrices. Gtfs also bind to other oral microbes, even those that do not naturally express Gtfs, thereby converting them into *de facto* glucan producers (7). In addition, *S. mutans* encodes several surface-associated glucan-binding proteins, GbpA, -B, -C, and -D. Together, the Gtfs, Gbps, and adhesive glucans serve as an integrated scaffold for sucrose-dependent biofilm formation central to this organism's cariogenicity by promoting the local accumulation of microbial cells while forming a diffusion-limiting polymeric matrix that protects the embedded bacteria (Fig. 2).

S. mutans also possesses multiple high-affinity surface adhesins that enable colonization even in the absence of sucrose. One of the most widely studied adhesins is the dual antigen I/II, also known as P1, SpaP, or PAc. This structurally complex multifunctional adhesin mediates bacterial attachment to the tooth's salivary pellicle via interactions with the host scavenger receptor glycoprotein GP340 or DMBT-1 (53–55). The AgI/II family adhesins also interact with other bacteria and host proteins such as fibronectin and collagen (53, 56). Relative to the wild type, a P1-deficient mutant demonstrates reduced binding to saliva or GP340-coated surfaces, aberrant biofilm formation, and reduced cariogenicity in a rat caries model (57, 58). In addition, P1 and WapA, another surface-localized adhesin, have been recently shown to form fibrillar amyloid aggregates (59). Amyloids are increasingly recognized as integral bacterial biofilm matrix components that interact with extracellular DNA (eDNA) and confer stability to the exopolysaccharide matrix (60). An *srtA* mutant lacking the only surface-anchoring sortase enzyme found in *S. mutans* is severely defective in biofilm development and does not produce amyloid, suggesting that fibril nucleation occurs at the cell surface (61). Treatment of *S. mutans* with known amyloid inhibitors inhibits biofilm formation via P1 and WapA-dependent mechanisms (59). In addition to P1 and WapA, a third amyloidogenic protein (SMU_63c) was recently identified. This secretory protein serves as an apparent negative regulator of biofilm cell density and genetic competence (59). Its production is associated with K^+ availability and influenced by growth phase (62).

Several cell envelope-associated proteins also contribute to *S. mutans* biofilm formation, including AtlA, RgpG, BrpA, and Psr. AtlA is an autolysin whose deficiency results in decreased autolysis and longer chain length and drastically reduces biofilm formation regardless of the carbohydrate used for growth (63). RgpG is the first enzyme of the biosynthetic pathway for rhamnose-containing glucose polymers, a major surface antigen of oral streptococci responsible for the different serotypes (64, 65). RgpG deficiency does not impair growth but leads to a pronounced reduction of cell surface antigens and major defects in cell morphology and cell division (65). RgpG-deficient mutants display long chains of swollen "squarish" dividing cells and form fewer biofilms irrespective of carbohydrate source. The BrpA and Psr proteins are members of the LytR-CpsA-Psr family of proteins that are widespread in Gram-positive bacteria (66–68). Deletion of *brpA* has little effect on growth but causes major impairment in biofilm formation. The *brpA* mutant is also less tolerant to acid and oxidative stresses and more susceptible to cell envelope antimicrobial agents, all of which likely contribute to its reduced biofilm phenotype. Psr also strongly influences *S. mutans* biofilm formation and acid tolerance, but unlike BrpA, Psr deficiency does not diminish oxidative stress tolerance or resistance to cell envelope antimicrobial agents. Both BrpA and Psr exhibit ligase functions for cell wall antigens, such as the rhamnose-

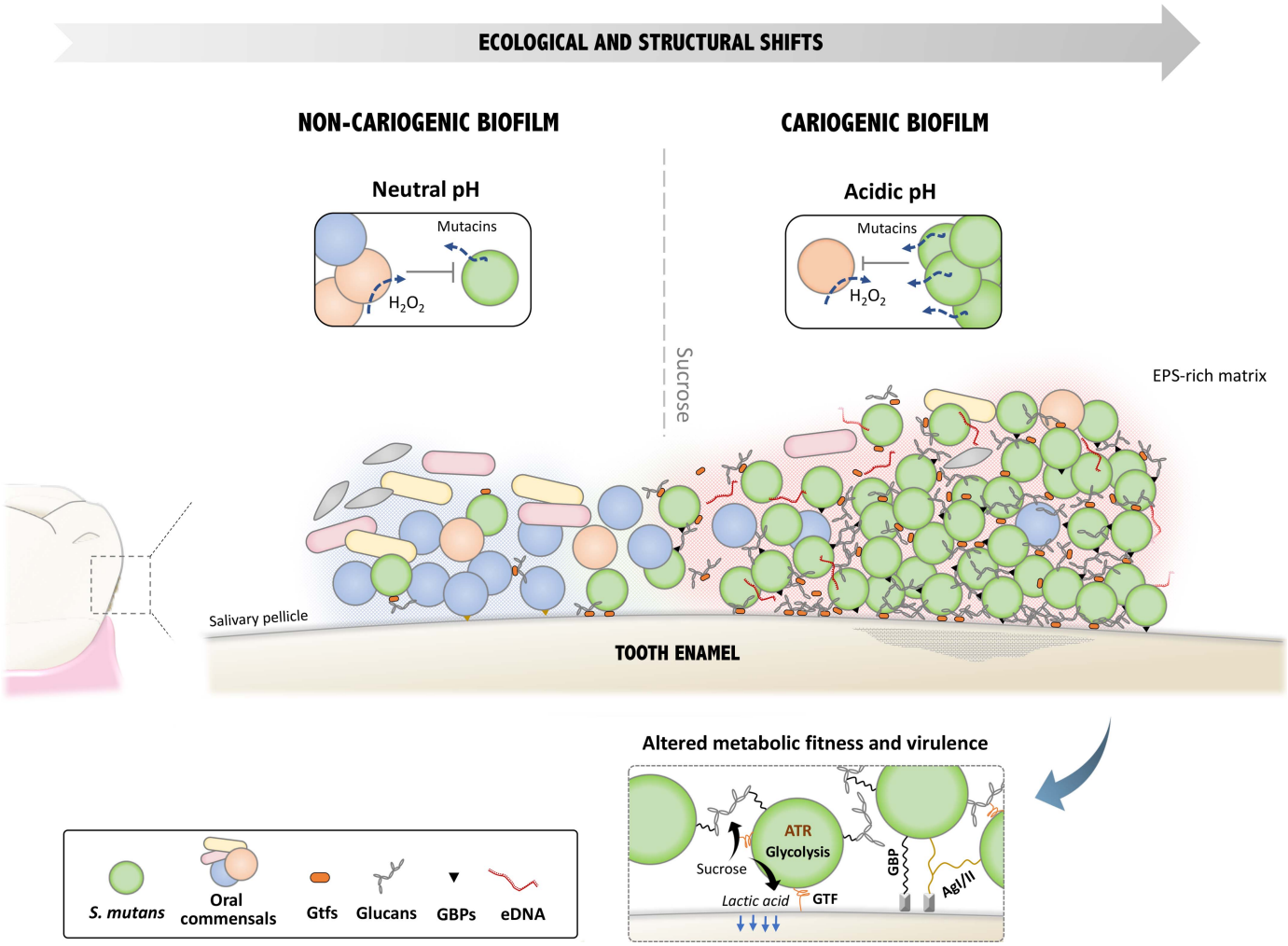

FIGURE 2 Biofilm formation and host-pathogen interactions in *S. mutans*. Early colonizers (e.g., *S. gordonii*, *Streptococcus mitis*, and *Actinomyces* spp. among others) attach to the tooth enamel via salivary proteins and start to form three-dimensional biofilms under noncariogenic conditions. At pH levels close to neutrality, production of H_2O_2 by peroxigenic bacteria and other antimicrobial products produced by oral commensals prevents the overgrowth of specific pathogens (e.g., *S. mutans*) on dental biofilms. Glycosyltransferases (GTFs) secreted by *S. mutans* adsorb onto the enamel pellicle or bacterial surfaces. In the presence of sucrose, GTFs catabolize sucrose to produce large amounts of insoluble and soluble glucans, which contribute to the buildup of a robust extracellular polysaccharide matrix (EPS), particularly insoluble components. The EPS matrix serves as an architectural scaffold for the biofilm structure, mediating tight adherence to the tooth enamel and bacteria, as glucans provide binding sites for *S. mutans* glucan-binding proteins (GBP) and other organisms. Extracellular DNA (eDNA) is another functional constituent of the oral biofilm matrix, forming nanofibers that connect cell to cell and cell to substratum and that contribute to biofilm structural integrity and stability. Continuous intake of sucrose by the host leads to a series of ecological and structural shifts that favor the growth of aciduric and highly acidogenic bacteria, such as *S. mutans*. These changes alter the oral biofilm metabolism so that copious amounts of organic acids are produced, contributing to a decrease in environmental pH. Once *S. mutans* becomes dominant, the secretion of large amounts of mutacins kills nearby competitors such as peroxigenic streptococci. The constant low-pH milieu surrounding the hydroxyapatite structure of the enamel leads to demineralization and initiates the carious process.

containing glucose polymers, and thereby globally impact cell envelope composition. Deficiency of either BrpA or Psr results in a reduction of cell wall antigens and their concurrent accumulation in cell-free culture medium (65). Finally, eDNA is another functional constituent of the *S. mutans* biofilm matrix; eDNA forms nanofibers that connect cells to one another and to the substratum contributing to bacterial adherence and biofilm structural integrity and stability (69).

STRESS TOLERANCE

The ability of *S. mutans* to adapt to sudden and substantial environmental changes within the dental plaque is a key attribute that contributes to its status as the major etiologic agent of dental caries. Fermentable carbohydrates consumed by the host provide a substrate for *S. mutans* and other lactic acid bacteria that ultimately results in the production of acidic end products that accumulate within the biofilm. To thrive at low pH values, *S. mutans* mounts the so-called acid tolerance response, a robust transcriptional and physiologic adaptation mechanism that encompasses the induction of pathways that contribute to cytoplasm buffering and changes in membrane fatty acid composition, ultimately protecting the cellular machinery from acid damage and contributing to the survival of the bacteria during stress (Fig. 3) (3, 6, 70). Collectively, the different cellular processes that constitute the acid tolerance response contribute to the ability of *S. mutans* to maintain an intracellular pH that is more alkaline than the surrounding environment (ΔpH) by about 0.5 to 1 pH unit (71). Environmental acidification also triggers *S. mutans* to modify the composition of the plasma membrane in a way that may alter proton permeability. These modifications are accomplished by increasing the proportion of monounsaturated fatty acids (both by incorporating exogenous fatty acids and by *de novo* synthesis) and by increasing the length of the carbon chains composing these membrane fatty acids (72). Inactivation of the gene responsible for biosynthesis of monounsaturated fatty acids in *S. mutans*, *fabM*, results in extreme sensitivity to low pH, inability to maintain ΔpH, and reduced virulence in a rat caries model (73, 74). In addition to changes in fatty acid composition, the phospholipid cardiolipin has also emerged as an important contributor to acid tolerance, because deletion of the cardiolipin synthase (*cls*) increased acid sensitivity (75).

Alkalinization of the cytoplasm occurs either by pumping protons out of the cell or through the generation of neutralizing molecules. In *S. mutans*, the membrane-bound F_1F_0-ATPase (F-ATPase) is the primary mechanism by which protons are extruded to maintain pH homeostasis. The *S. mutans* F-ATPase is transcriptionally induced by low pH values and has an optimal pH of 6.0, which is lower than that of most streptococci associated with oral health (76, 77). Some oral streptococci utilize the urease enzyme (e.g., *Streptococcus salivarius*) or the arginine deiminase system (e.g., *Streptococcus gordonii*) to produce the neutralizing molecules ammonia and CO_2 in order to cope with acid stress. Although these pathways are absent in *S. mutans*, the agmatine deiminase system (AgDS), analogous to the arginine deiminase system, is present in this species (78, 79). The AgDS converts agmatine, a decarboxylated derivative of arginine found in dental plaque, to ammonia, CO_2, putrescine, and ATP. Though the AgDS does not appear to have a considerable impact on environmental alkalinization, the ammonia generated internally may contribute to neutralization of the cytoplasmic pH, while the ATP generated can be used to fuel proton extrusion via the F-ATPase (80). Malolactic fermentation converts malate, an acid commonly found in wine and in fruits such as apples, to the less acidic lactate and to CO_2. The CO_2 product can then be used for cytoplasmic neutralization by conversion to bicarbonate via carbonic anhydrase. In *S. mutans*, transcription of the genes encoding the malolactic enzyme and permease is acid-inducible, and malolactic fermentation activity was found to be optimal at an extracellular pH of 4.0 (81). Most importantly, malate was shown to protect *S. mutans* against acid killing and was associated with maintenance of ATP pools during starvation (82).

While the dental plaque environment was initially thought to be anaerobic, it is now known that the oral microbial community has a high capacity to reduce oxygen, resulting in the generation of toxic reactive oxygen species (83). Most *S. mutans* strains are susceptible to exposure to H_2O_2, which can be generated by metabolism of other species within the dental plaque, such as peroxigenic streptococci, or is contained as a component of oral hygiene and tooth bleaching products. Exposure to high levels of H_2O_2, and its more damaging breakdown products hydroxyl radical and superoxide anion, can rapidly cause irreversible cellular damage by triggering mismetallation of enzymes, by damaging proteins through oxidation of sulfurous amino acids and metal-binding sites, and by disturbing DNA integrity (84). Although oral streptococci lack catalase, *S. mutans* utilizes a number of scavenging and protective systems to prevent the accumulation of toxic reactive oxygen species. Among these are a manganese-dependent

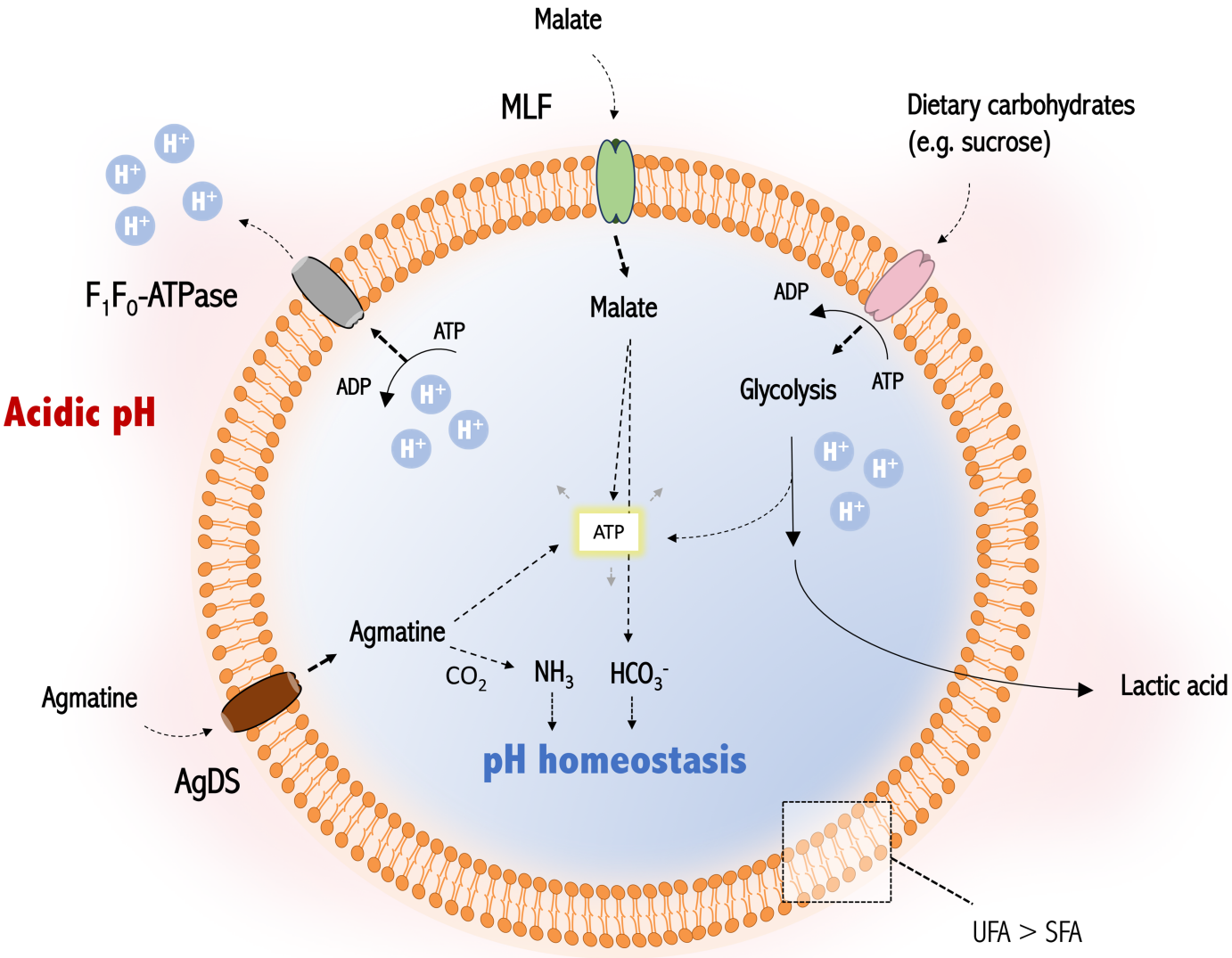

FIGURE 3 Acid stress tolerance mechanisms of *S. mutans*. The ability of *S. mutans* to catalyze fermentable dietary carbohydrates into organic acids can promptly drop the environmental pH. Exposure to sublethal low pH triggers an acid-adaptive response known as ATR (acid tolerance response), which is a robust transcriptional and physiologic adaptation mechanism for pH homeostasis through alteration of proton permeability, generation of neutralizing molecules, and changes in membrane fatty acid composition. The membrane F_1F_0-ATPase (F-ATPase) is transcriptionally induced by low pH and serves as the primary mechanism by which protons are extruded to maintain pH homeostasis. The modifications in membrane composition refer to an increase in the proportion of monounsaturated fatty acids (UFA) over saturated fatty acids (SFA) and in the length of the carbon chains composing the membrane fatty acids. Production of neutralizing molecules, such as ammonia and CO_2, is also an important way to cope with acid stress. In *S. mutans*, the agmatine deiminase system (AgDS) converts agmatine, a decarboxylated derivative of arginine found in dental plaque, to ammonia, CO_2, putrescine, and ATP. The ammonia generated internally may contribute to cytoplasmic buffering, while the ATP generated can be used to fuel proton extrusion via the F-ATPase. A decrease in environmental pH also triggers activation of malolactic fermentation (MLF), which converts malate to the less acidic lactate and to CO_2. The CO_2 product can then be used for cytoplasmic neutralization by conversion to bicarbonate (HCO_3^-) via carbonic anhydrase.

superoxide dismutase, an alkyl hydroperoxidase reductase, a thioredoxin reductase, and a glutaredoxin system (GshA/B/R) (85). Tight control of free iron in the cell is an important aspect of minimizing exposure to reactive oxygen species, because free radical formation is a direct result of the Fenton chemistry, when H_2O_2 comes into contact with ferrous iron. For this reason, the iron binding protein Dpr is a critical mediator of oxidative stress exposure in *S. mutans* (86, 87). Finally, the Spx regulators, SpxA1 and SpxA2, are responsible for the transcriptional activation of virtually every major oxidative stress response gene in *S. mutans* (85, 88). Not surprisingly, *spx* deletion strains were ill-equipped to tolerate oxidative challenges and caused fewer sulcal caries lesions in rats fed a highly cariogenic diet (85, 89, 90).

SIGNALING PATHWAYS

Two-Component Signal Transduction Systems (TCSTSs)

TCSTSs play important roles in bacterial adaptation, survival, and virulence by sensing changes in the environment and altering the expression of specific sets of genes to mount coordinated responses to environmental stimuli. In TCSTSs, a signal is sent by phosphotransfer between histidine senor kinases and their cognate response regulators, triggering the response regulators to dimerize and bind to conserved DNA motifs, ultimately tuning cellular functions such as biofilm formation, stress tolerance, and nutrient uptake (91). The genome of the type strain UA159 encodes 14 TCSTSs and one orphan response regulator with no genetic link to a histidine kinase (92). Among those, ComDE, LevRS, VicRK, and the orphan CovR regulator have been studied in some detail and shown to coordinate the expression of a number of virulence attributes. The LevRS system, which is important for management of carbohydrate metabolism, was introduced in the "Carbohydrate Metabolism" session, whereas the ComDE system, which is involved in the regulation of bacteriocin production and competence, is discussed in the next paragraph. VicRK influences acid and oxidative stress responses and competence and is the only TCSTS essential in *S. mutans* (93, 94). Several studies have proposed phosphotransfer cross talk between VicRK and the LiaFSR TCSTS, which contributes to surface adhesion, mutacin production, and the ability to tolerate environmental stresses, including cell envelope damage and heat shock (95–98). In addition, VicRK and CovR directly regulate genes implicated in the synthesis of and interaction with extracellular polysaccharides (7, 99). For example, transcription of *gbpB* is positively regulated by VicR (100, 101), while *gtfB*, *gtfC*, and *gbpC* are repressed by CovR (99).

Quorum Sensing (QS)

QS systems are communication networks that enable microbes to sense and respond to environmental conditions such as nutrient availability and population density, because signaling molecules naturally accumulate along with bacterial density. In Gram-positive bacteria, QS is coordinated by peptide pheromones, which serve as extracellular signaling molecules that trigger changes in gene expression and, ultimately, activation of a coordinated response by the population (102–104). In *S. mutans*, QS systems have evolved to regulate production of bacteriocins (known in *S. mutans* as mutacins), which are peptide antibiotics used in defense against other oral microbes (105–107) and in competence (natural transformation), a transient state in which the organism is primed to take up foreign DNA (Fig. 4). The competence-stimulating peptide signaling molecule (CSP) (also called the mutacin-inducing peptide) is encoded by *comC*. Once CSP has been secreted and processed, it is recognized by the TCSTS ComDE (104, 108, 109). Activated (phosphorylated) ComE regulates mutacin production by recognizing a conserved sequence in the promoter region of its target genes (95, 110, 111). Also sharing regulatory links with ComDE in *S. mutans* is another TCSTS, CiaRH, which contributes to biofilm formation, acid tolerance, and entry into competence, possibly by interacting with CSP (112). Among the consequences of ComE activation is the stimulation of *comRS*, the QS pathway directly responsible for competence activation via the alternative sigma factor ComX (also called SigX) (113, 114). Once exported to the extracellular space, the prepeptide ComS is presumably processed into the peptide pheromone XIP (*comX*- or *sigX*-inducing peptide) (114, 115). When imported back into the cell via the oligopeptide permease system, XIP is sensed by ComR, which binds to conserved inverted repeat sequences upstream of both *comX* and *comS*, thereby causing auto-induction of the ComRS pathway and activation of ComX-regulated genes. It is worth noting that while all *S. mutans* isolates sequenced to date possess a functional ComRS-ComX system, not all strains harbor a complete ComCDE pathway, nor are all isolates equally competent (15).

The mutacins produced by *S. mutans* are categorized as either lantibiotics (broadly active against Gram-positive bacteria) or nonlantibiotics (most active against closely related species), and production of these molecules is strain specific (116). Mutacins provide a competitive advantage to *S. mutans* by inhibiting the growth

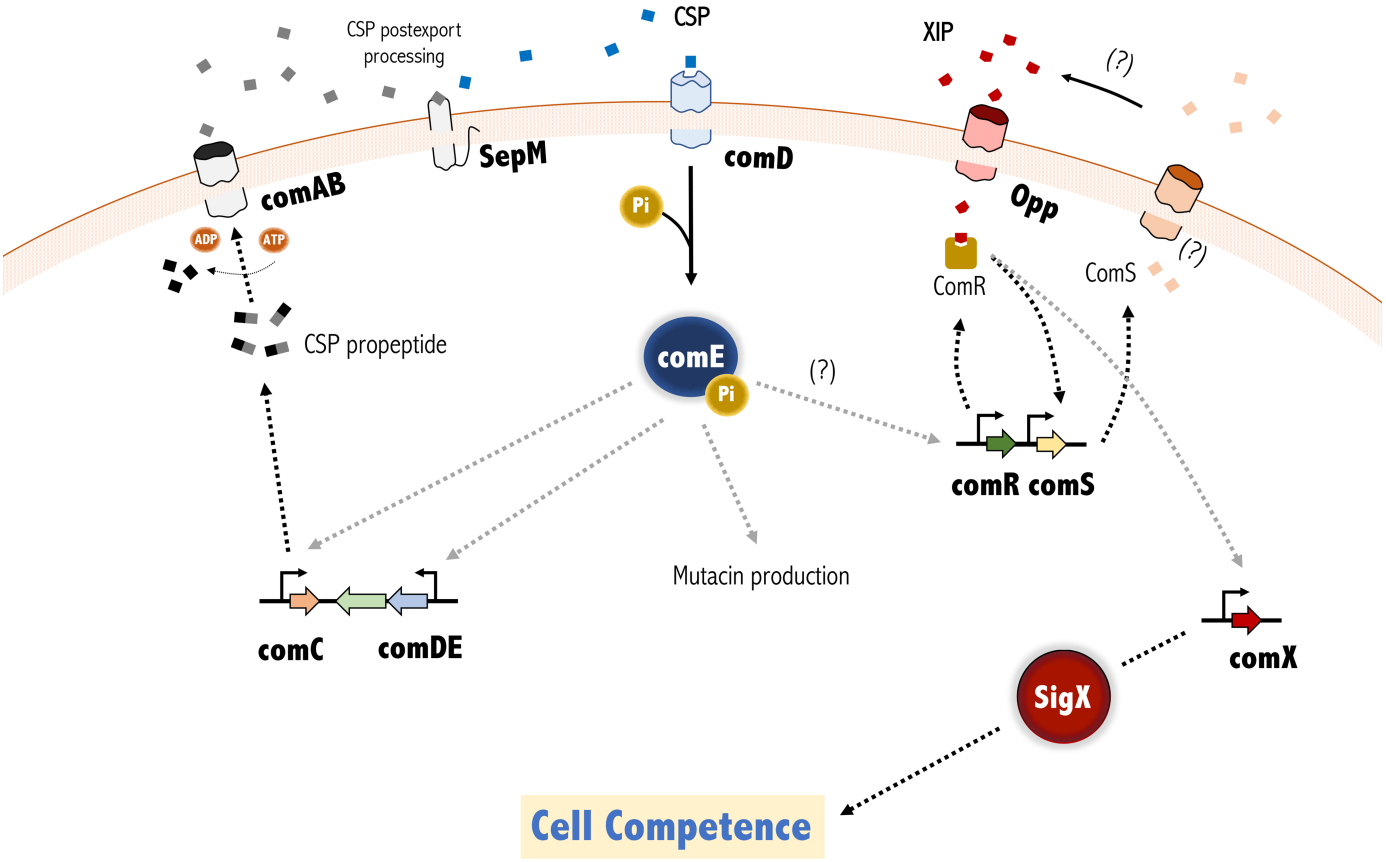

FIGURE 4 Quorum-sensing systems involved in the regulation of bacteriocin production and competence in *S. mutans*. Cell density, nutrient availability, and other environmental conditions induce the expression of *comC*, a gene encoding the competence-stimulating peptide (CSP) precursor. The CSP propeptide is cleaved off inside the cell and exported through a specific ABC transporter encoded by *comAB*. In the extracellular environment, CSP then undergoes a final postexport processing mediated by the SepM protease. Upon reaching a certain threshold, mature CSP is recognized by the two-component system ComDE, triggering a phosphorylation cascade. Activated (phosphorylated) ComE activates transcription of (i) *comC* and *comDE*, creating a positive feedback loop, (ii) genes involved in mutacin production, and (iii) by a yet-to-be-determined mechanism, *comRS*, the quorum-sensing pathway directly responsible for competence activation via the alternative sigma factor ComX. Once exported to the extracellular milieu, the prepeptide ComS is processed into the peptide pheromone XIP (*comX*- or *sigX*-inducing peptide). XIP is transported back into the cell via the oligopeptide permease system (Opp) and sensed by the Rgg-type regulator ComR. Activated ComR binds to both *comX* and *comS* promoters, thereby causing auto-induction of ComRS and activation of late (ComX-regulated) competence genes.

of neighboring bacterial species (117, 118). A close relationship exists between competence induction and mutacin production, because a threshold concentration of CSP activates ComDE to result in increased mutacin production (95, 111). Adding to the layers of regulation of mutacin production are two LytTR regulatory systems, HdrRM and BrsRM, which include a membrane-bound inhibitor protein that prompts the activity of the transcriptional regulator (119, 120). In this case, an overlap in the regulatory systems that govern mutacin production and competence is again observed because LytTR systems also induce competence through ComX (120, 121). A complete understanding of the environmental conditions that trigger these regulators and hence influence mutacin production is yet to be achieved.

Another *S. mutans* QS mechanism employs the autoinducer-2 (AI-2), the collective name for a group of furanones formed as a by-product of LuxS-mediated

methyl metabolism (122). The lack of species specificity of AI-2 signaling molecules, which are produced by both Gram-positive and Gram-negative organisms, facilitates interspecies communication as population growth contributes to accumulation of AI-2. Deficiency of LuxS reduces the ability of *S. mutans* to tolerate acid and oxidative stress and to form biofilms, especially during growth in sucrose (123, 124). In agreement with those phenotypes, LuxS deficiency causes alterations in expression of a large number of genes, including those with known roles in stress tolerance response and biofilm formation (123–125). Addition of synthetic AI-2 to the growth medium can restore the phenotypes of LuxS mutants with regard to the stress tolerance response, biofilm formation, and the expression of selected genes (124, 125). To date, however, a specific receptor for AI-2 has yet to be identified (125).

Regulatory Nucleotides

The regulatory nucleotides (p)ppGpp and cyclic-di-AMP have also been shown to modulate biofilm formation and stress responses in *S. mutans* (126–128). Guanosine tetraphosphate (ppGpp) and guanosine pentaphosphate (pppGpp), collectively called (p)ppGpp, are the effector molecules of a conserved stress response referred to as the stringent response (129). Three (p)ppGpp-metabolizing enzymes have been identified in *S. mutans*: RelA is a bifunctional synthetase/hydrolase contributing to metabolism of (p)ppGpp, while two small alarmone synthetase enzymes, RelP and RelQ, harbor only the synthetase domain (130). In *S. mutans*, changes in (p)ppGpp pools affect biofilm formation, carbohydrate metabolism, and stress tolerance, suggesting an overlap between circuits that govern general stress tolerance and biofilm formation. While the mechanism is not completely understood, (p)ppGpp pools also modulate competence signaling through interactions with an ABC-type transporter, RcrRPQ, and at least one peptide encoded within *rcrQ* (126, 131). Cyclic di-AMP is an emerging second messenger in bacteria that has been shown to play important roles in bacterial fitness and virulence (132). In *S. mutans*, increased cyclic-di-AMP levels promoted biofilm formation through interactions with the VicRK-*gtfB* network that ultimately resulted in in the transcriptional activation of *gtfB* (128).

INTERSPECIES AND CROSS-KINGDOM INTERACTIONS

Interactions between *S. mutans* and certain members of the dental plaque community have been documented and have recently been shown to exert a major influence on the development and pathogenicity of plaque. These interactions can be synergistic, i.e., promote *S. mutans* growth, or antagonistic, i.e., inhibitory to *S. mutans*. A classic example of an antagonistic interaction occurs between *S. mutans* and members of the mitis streptococci group such as *Streptococcus sanguinis* and *S. gordonii*. Specifically, several members of the mitis group secrete millimolar concentrations of H_2O_2, which can be highly inhibitory to *S. mutans* (118). On the other hand, strains of *S. mutans* not only produce mutacins that are specific against mitis streptococci, but most are also generally more aciduric than species from the mitis group. This antagonistic relationship becomes more evident in dental plaque, whereby there is an inverse association between the abundance of oral commensal streptococci such as *S. sanguinis* and *S. gordonii* (abundant in healthy plaque) and that of *S. mutans* (abundant in caries lesions) (133) (Fig. 2).

While production of lactic acid by *S. mutans* is inhibitory to the growth of many acid-sensitive oral commensals, lactic acid serves as a carbon source for *Veillonella* spp., a prevalent genus in the oral cavity (134). Thus, it is thought that *Veillonella* may act as an "acid sink," preventing the dental biofilm from reaching extremely low pH values that even aciduric organisms such as *S. mutans* may not be able to survive. This nutritional mutualism may explain the close association of *Veillonella* with streptococci in the oral cavity, which excrete lactate as a waste product of carbohydrate fermentation. In dual-species biofilms, *S. mutans* and *Veillonella parvula* form distinctive structures and display increased resistance to chlorhexidine and other antimicrobials when compared to single-species biofilms (135, 136). Using a three-species mixed culture, Liu et al. showed that the presence of *V. parvula* negated the growth inhibition of *S. mutans* caused by peroxigenic *S. gordonii* (137).

In addition to bacteria, the oral cavity is also colonized by yeasts. *Candida albicans* in particular is frequently found in the oral mucosa. Interestingly, *C. albicans* is often associated with *S. mutans* and detected in high numbers in cases of early childhood caries (138). Recent studies have shown that the *S. mutans* GtfB enzyme binds to mannan receptors on the *C. albicans* surface, leading to enhanced adherence and biofilm accumulation by *C. albicans* and thereby enhancing biofilm formation and cariogenicity (139, 140). At the same time, *C. albicans* synthesizes farnesol, which enhances extracellular polysaccharide production by *S. mutans* (52). In a rat caries model, coinfection with *S. mutans* and *C. albicans* led to higher levels of microbial carriage in

plaque biofilms and significantly increased the cariogenic potential of dental plaque, resulting in aggressive disease onset and rampant caries lesions (140).

S. MUTANS AS A MODEL ORGANISM: SHIFTING LONG-STANDING PARADIGMS

Key advances in the field of bacteriology have come from studies with *Escherichia coli* and *Bacillus subtilis*, which represent the Gram-negative and Gram-positive bacterial paradigms, respectively. Despite the important contributions of these two species to bacteriology research, no model organism is a perfect representative of other species. In the case of Gram-positive organisms, *B. subtilis* is a free-living sporulating organism found in soil and plants but not in humans. Conversely, *S. mutans* is an obligate human pathogen with a biofilm-dependent lifestyle. It does not come as a surprise that a greater overlap in the mechanisms of gene regulation and metabolic pathways are observed between *S. mutans* and related Gram-positive pathogens that share a host-associated lifestyle. Such organisms, like *S. mutans*, generally have compact genomes with low GC contents. Like *E. coli* and *B. subtilis*, *S. mutans* is highly amenable to genetic manipulation and is therefore also easy to study and control in the laboratory setting. Among closely related streptococci, it has the most complete and sophisticated set of genetic tools that can be used in combination with simple and efficient *in vitro* and *in vivo* models to enhance our current understanding of *S. mutans* biology and beyond (141). As a result, a number of *S. mutans* investigations have challenged long-standing bacterial dogmas that were established based on work conducted with *E. coli* and *B. subtilis*. One such example is the signal recognition particle (SRP) pathway involved in cotranslational protein translocation (142). The first clue that protein transport in *S. mutans* differs from the established *E. coli* model came from the identification of an acid-sensitive transposon mutant in which *ffh* encoding the 54-kilodalton homolog of the eukaryotic SRP was insertionally inactivated. Subsequently, all three conserved bacterial elements of the cotranslational translocation SRP pathway, including the particle components Ffh and small 4.5S cytoplasmic RNA, along with the membrane-associated SRP receptor FtsY, were proven dispensable in *S. mutans*. This was noteworthy because the SRP pathway was long believed to be essential for viability in all living cells. An explanation for this surprise lay in the presence in *S. mutans* of two paralogs of the YidC/Oxa/Alb family of membrane-localized chaperone insertases found in bacteria, mitochondria, and chloroplasts. Unlike Gram-negative bacteria, most Gram-positive organisms possess two, if not more, YidC homologs. Another almost universal distinguishing feature between Gram-positive and Gram-negative bacteria, also discovered in *S. mutans*, is the SRP accessory factor YlxM, which interacts with both Ffh and scRNA and influences the GTPase activity of the Ffh/FtsY heterodimer, which is necessary for pathway component recycling once ribosome/nascent protein chain complexes are delivered to the membrane-localized SecYEG translocon (143). Another example occurred during the initial characterization of the enzyme responsible for the synthesis of (p)ppGpp in *S. mutans*, the effector molecule of the bacterial stringent response (see "Signaling Pathways" section). Before the work with *S. mutans*, the bifunctional RelA enzyme (also known as Rsh or Rel) was considered the sole enzymatic source of (p)ppGpp synthesis and degradation in all *Firmicutes*. The serendipitous finding that deletion of *relA* did not abolish (p)ppGpp production in *S. mutans* led to the discovery of two additional enzymes, RelP and RelQ, capable of synthesizing (p)ppGpp (130). Subsequent bioinformatics and genetic analysis revealed that these enzymes are ubiquitous in *Firmicutes*, and they were eventually found in other bacterial species.

S. MUTANS AS A THERAPEUTIC TARGET IN CARIES PREVENTION

While caries is a polymicrobial disease, selective targeting of *S. mutans* in dental biofilms is viewed as a suitable approach for its prevention. This is mainly because the synthesis of insoluble glucans from sucrose by *S. mutans* is central for the formation of a stable biofilm matrix that facilitates bacterial colonization of the tooth surface and, at the same time, serves as a diffusion barrier helping to maintain the acidic milieu within which cariogenic bacteria thrive (144). One of the current lines of thought in dental caries prevention is that it may be possible to halt the development of cariogenic biofilms by selectively targeting *S. mutans* such that the oral microbiome associated with health is not disturbed. Most of the early research conducted in the late 1960s into the 1980s focused on the development of anti-*S. mutans* vaccines. Despite some recent successes in animal models with vaccines containing or encoding single or a combination of antigens (145–148), emphasis on an immunoprophylactic approach has slowed somewhat due to the many challenges in obtaining long-term salivary IgA responses, safety concerns with antistreptococci antibody cross-reactivity, and providing species-specific protec-

tion without disrupting related beneficial commensal organisms. Over the past 2 decades or more, a number of natural products, such as propolis (149), curcumin (150), cranberry (151), and green tea extracts (152) among many others, have been shown to be effective against *S. mutans* biofilms and to prevent dental caries, although to date, none of these products has proven to be selective toward *S. mutans*. More recently, antimicrobial peptides (AMPs), small molecules, and probiotics have emerged as promising new approaches for the development of novel anticaries strategies that specifically target *S. mutans*. While AMPs kill bacteria indiscriminately, the selective killing of *S. mutans* by AMPs has been achieved by creating a synthetic peptide that combines a large-spectrum AMP with the *S. mutans*-specific CSP (153, 154). Phase 2 clinical trials recently showed that a single varnish application of one such peptide achieved significant reductions of *S. mutans* in the oral cavity (155). More recently, a small molecule derived from 2-aminotriazole (designated 3F1) was also shown to selectively disperse *S. mutans* biofilms (156). Most importantly, 3F1 was shown to reduce caries without disturbing the oral microbiome in a rat caries model. An alternative approach to combat *S. mutans* and therefore prevent dental caries may be the use of alkalinogenic bacteria as a probiotic or the use of substrates for such alkalinogenic activities, e.g., arginine, as a prebiotic. This is because some oral bacteria can neutralize plaque pH by producing large quantities of ammonia by metabolizing arginine or urea, thereby creating an environment favorable to health-associated bacteria (157). A promising candidate is a highly arginolytic streptococcal strain, designated A12, isolated from supragingival plaque of a caries-free individual (158). Not only did the A12 strain neutralize acid by metabolizing arginine, but it was also shown to kill *S. mutans* by producing H_2O_2 and to interfere with signaling pathways that control bacteriocin production.

FUTURE PERSPECTIVES

Evidence accumulated over many decades has clearly shown that *S. mutans* is a major agent in dental caries vis-à-vis its capacity to orchestrate changes in the plaque microbiome via EPS and acid production. Thus, continued efforts to elucidate how *S. mutans* senses and responds to environmental cues through interconnected circuits that govern stress tolerance and biofilm formation can facilitate the identification of new targets for caries treatment and prevention. As the biology of *S. mutans* continues to be unraveled, a better understanding of the consequences of genomic and phenotypic heterogeneity among strains is an important area for development. Specifically, how *S. mutans* isolates with different properties contribute to the different stages of disease through synergistic or antagonistic interactions with the microbiome is a largely unexplored area. Future investigations should also consider the role of certain strains of *S. mutans* in systemic infections.

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