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JOURNAL OF BACTERIOLOGY, July 2007, p. 5049–5059 Vol. 189, No. 14
0021-9193/07/$08.00+0 doi:10.1128/JB.00338-07
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Global Transcriptional Analysis of *Streptococcus mutans* Sugar Transporters Using Microarrays▽†

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Received 7 March 2007/Accepted 4 May 2007

The transport of carbohydrates by *Streptococcus mutans* is accomplished by the phosphoenolpyruvate-phosphotransferase system (PTS) and ATP-binding cassette (ABC) transporters. To undertake a global transcriptional analysis of all *S. mutans* sugar transporters simultaneously, we used a whole-genome expression microarray. Global transcription profiles of *S. mutans* UA159 were determined for several monosaccharides (glucose, fructose, galactose, and mannose), disaccharides (sucrose, lactose, maltose, and trehalose), a β-glucoside (cellobiose), oligosaccharides (raffinose, stachyose, and maltotriose), and a sugar alcohol (mannitol). The results revealed that PTSs were responsible for transport of monosaccharides, disaccharides, β-glucosides, and sugar alcohol. Six PTSs were transcribed only if a specific sugar was present in the growth medium; thus, they were regulated at the transcriptional level. These included transporters for fructose, lactose, cellobiose, and trehalose and two transporters for mannitol. Three PTSs were repressed under all conditions tested. Interestingly, five PTSs were always highly expressed regardless of the sugar source used, presumably suggesting their availability for immediate uptake of most common dietary sugars (glucose, fructose, maltose, and sucrose). The ABC transporters were found to be specific for oligosaccharides, raffinose, stachyose, and isomaltosaccharides. Compared to the PTSs, the ABC transporters showed higher transcription under several tested conditions, suggesting that they might be transporting multiple substrates.

Numerous studies have implicated *Streptococcus mutans* as the principal causative agent of human dental caries. It is well known that host diet is important for *S. mutans* cariogenicity, and sugar metabolism of this bacterium plays a key role in the formation of caries. *S. mutans* is able to metabolize a wide range of carbohydrates that may originate from dietary sources or from host macromolecules. If the diet is rich in sugars, especially sucrose, the end product of sugar metabolism is mostly lactic acid that can lead to demineralization of tooth enamel. Therefore, sugar transport and metabolism by this bacterium are directly related to the onset and development of dental caries.

In bacteria, sugar substrates are taken up by ATP-binding cassette (ABC) transporters, galactoside-pentose hexuronide (GPH) translocators, and, most commonly, phosphoenolpyruvate-sugar phosphotransferase systems (PTSs). The bacterial PTSs are responsible for the binding, transmembrane transport, and phosphorylation of numerous sugar substrates. These systems are also involved in the regulation of a variety of metabolic and transcriptional processes (29, 37). The PTS consists of two nonspecific energy-coupling components, enzyme I (EI) and a heat-stable protein (HPr), as well as several sugar-specific multiprotein permeases known as EII. In most cases, EIIA and EIIB are located in the cytoplasm, while EIIC acts as a membrane channel (37). Fourteen PTS systems have been found in *S. mutans* UA159 based on sequence analysis (4).

The ABC transporters are widespread in bacteria and show a common global organization with three types of molecular components. Typically in gram-positive bacteria, an ABC transporter consists of two integral membrane proteins (permeases), two peripheral membrane proteins that bind and hydrolyze ATP, and an extracytoplasmic solute-binding receptor that can be a lipoprotein anchored to the external surface of the cytoplasmic membrane or a cell surface-associated protein that is bound to the external membrane surface via electrostatic interactions. Four sugar ABC transport systems exist in *S. mutans* (4), including the well-described multiple-sugar metabolism (MSM) system (28, 33, 34).

GPH translocators are electrochemical potential-driven transporters and they catalyze the uptake of sugars in symport with monovalent cations (H^+, Na^+, or Li^+). In different bacterial species, these transporters exhibit specificity for β-galactosides, lactose, and both anomers of galactose (26). Most members of the GPH family are regulated by the PTS (24). PTS transport of galactose has not been reported to occur in *S. mutans*, and it is possible that this sugar is transported via an ABC or a GPH transporter.

In this study, we analyzed differential transcription profiles of *S. mutans* genes involved in sugar transport, metabolism, and regulation following growth with 13 different sugars: glucose, fructose, mannose, sucrose, lactose, galactose, maltose, maltotriose, trehalose, raffinose, stachyose, cellobiose, and mannitol. Although sugar transport and metabolism by *S. mutans* have been extensively studied, this is the first study in which transcriptional analysis of all sugar transporters of this organism was performed simultaneously using whole-genome microarrays.

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† Supplemental material for this article may be found at http://jb.asm.org/.

▽ Published ahead of print on 11 May 2007.

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MATERIALS AND METHODS

Bacterial strain and media. The strain used in this study was *S. mutans* UA159. Bacterial cultures were routinely propagated at 37°C in Todd-Hewitt broth and 0.3% yeast extract (THY). For RNA isolation, cultures were grown overnight in THY, pelleted, washed two times in phosphate-buffered saline, and resuspended in defined medium (FMC) (35) supplemented with 0.5% carbohydrate (~99 to 99.5% purity and from Sigma, except for stachyose and maltotriose, which were >98% and >96% purity, respectively, and purchased from Fluka). Dextranase (1,6-α-D-glucan 6-glucanohydrolase) from *Penicillium* sp. (Worthington) was added, when indicated, to the glucose- and sucrose-grown cultures of UA159 at a concentration of 10 U/ml.

Growth curves of *S. mutans* UA159 were generated using a Bioscreen C analyzer, version 2.4 (Oy Growth Curves AB Ltd., Finland), which kinetically and simultaneously measured the development of turbidity in multiple cultures. The cultures were incubated at 37°C for 24 h in defined medium (FMC) supplemented with the appropriate carbohydrate. The wide-band 420- to 580-nm filter, which is rather insensitive to color changes of the medium, was used to detect the optical density of the cultures. Growth of *S. mutans* in most of the studied sugars showed similar rates, with the exception of growth in cellobiose, mannose, and mannitol. However, growth of UA159 in each of these three sugars was similar to one another, and so the microarray results were compared to each other and to those for the glucose-grown samples. Growth of UA159 on galactose was considerably slower than that in any other growth condition.

RNA isolation. Each *S. mutans* culture was inoculated from a single colony in 5 ml of THY and incubated at 37°C for approximately 16 h. The culture was pelleted and washed three times with defined medium (FMC). The cells were then resuspended in 5 ml of FMC containing 0.5% of the desired sugar. This culture was transferred to 45 ml of prewarmed FMC also supplemented with 0.5% sugar and grown at 37°C to an optical density at 600 nm of 0.62 to 0.65. The pellet was collected by centrifugation (6,000 rpm) at 4°C for 10 minutes. One half of the cell pellet was used for RNA isolation and processed as four samples. The samples were resuspended in 400 μl of ice cold RNA_{wiz} (Ambion) and disrupted with 250 μl of 0.1-mm zirconia beads using a bead beater. Cells were subjected to three consecutive 1-minute homogenizations. Two sample lysates were pooled and purified using the Ambion RiboPure-Bacteria kit according to the provided protocol. Isolated RNA was treated twice with DNase I (Ambion) to remove traces of chromosomal DNA. After the treatment, RNA samples were cleaned with the QIAGEN RNeasy MinElute cleanup kit. The purity, concentration, and quality of RNA samples were confirmed by PCR, NanoDrop spectrophotometer measurements, and gel electrophoresis.

cDNA synthesis. For cDNA synthesis, 15 μg of RNA was mixed with 1.25 μg of random primers and incubated at 70°C for 10 min and then at 25°C for 10 min. The reverse transcription was performed in 1× First Strand buffer supplemented with 10 mM dithiothreitol, 0.5 mM deoxynucleoside triphosphate mix, 60 U of RNase inhibitor (RNAseOut; Invitrogen), and 1,500 U of SuperScript II reverse transcriptase (Invitrogen) in a final volume of 60 μl. The reaction mixture was incubated at 25°C for 10 min, 37°C for 1 h, and 42°C for 1 h. Reverse transcription was terminated by a 10-min incubation at 70°C. To remove residual RNA, the cDNA samples were treated with 0.2 N NaOH at 65°C for 30 min and then neutralized with 0.2 N HCl before being purified with the QIAGEN MinElute PCR purification kit. Synthesized cDNA was quantified using a NanoDrop spectrophotometer. cDNA was fragmented with Roche DNase I (0.06 U per 1 μg of cDNA) at 37°C for 10 min. Inactivation of the enzyme was performed at 98°C for 10 min. The quality and size (50 to 200 nucleotides) of the fragmented cDNA were evaluated using the Bioanalyzer 6000 (Agilent Technologies), followed by labeling with biotin-ddUTP using the BioArray terminal labeling kit (Enzo).

Microarray. A whole-genome custom GeneChip antisense expression microarray was designed in collaboration with Affymetrix (Santa Clara, CA). On the oligonucleotide array, each gene of *S. mutans* was represented by 17 25-mer oligonucleotides that were designed to be complementary to the target sequence. These oligonucleotides served as unique, sequence-specific detectors (termed perfect-match [PM] probes). An additional control element on these arrays was the use of 17 mismatch (MM) control probes for each gene. These probes were designed to be identical to their PM partners except for a single base difference in the central position. The presence of the mismatched oligonucleotide allows cross-hybridization and local background to be estimated and subtracted from the PM signal. A probe pair is called positive when the intensity of the PM probe cell is significantly greater than that of the corresponding MM probe cell, and a probe pair is called negative if the situation is reversed. All probes were designed using Affymetrix probe selection software and were selected to ensure minimal cross-hybridization. Each probe was tiled to the array in approximately one million copies.

Hybridization, washing, and scanning of the microarray chips were performed according to the procedures described by Affymetrix. The hybridization solution contained 100 mM MES (morpholineethanesulfonic acid), 1 M NaCl, 20 mM EDTA, and 0.01% Tween 20, pH 6.6 (referred to as 1× MES). In addition, the solution contained 0.1 mg ml^{-1} herring sperm DNA, 0.5 mg ml^{-1} bovine serum albumin (BSA), 7.8% dimethyl sulfoxide, and 50 pM of the B2 control oligonucleotide. Samples were placed in the array cartridge, and hybridization was carried out at 47°C for 16 h with mixing on a rotisserie at 60 rpm. The hybridization solution was removed and the array washed and stained in the Fluidics station using the FlexMidi_euk2v3_450 protocol with modifications for the *Pseudomonas aeruginosa* array (as recommended in the technical manual from Affymetrix). To enhance the signals, a solution of 10 μg ml^{-1} streptavidin and 2 mg ml^{-1} BSA in 1× MES was used as the first staining solution. After the streptavidin solution was removed, an antibody mix was added as the second stain, containing 0.1 mg ml^{-1} goat immunoglobulin G, 5 μg ml^{-1} antistreptavidin antibody, and 2 mg ml^{-1} BSA in 1× MES. Nucleic acid was fluorescently labeled by incubation with 10 μg ml^{-1} streptavidin-phycoerythrin (Molecular Probes, Eugene, OR) and 2 mg ml^{-1} BSA in 1× MES. The arrays were read at 570 nm with a resolution of 0.5 μm using a 7G laser scanner (Affymetrix, Santa Clara, CA).

The GeneChip operating software (GCOS) version 1.4 analysis program (Affymetrix, Santa Clara, CA) was used for the analysis of gene expression and expression clustering. The data were compared using GCOS batch analyses. Normalization of all probe sets was done by GCOS. The software computes a normalization value so that trimmed mean $signal_{baseline}$ = normalization value × trimmed mean $signal_{experiment}$. The cutoff score for the analysis was twofold. Due to a limitation of the software, differences lower than twofold could be inconsistent and therefore not significant.

RT-PCR. Quantification of the specific transcript was accomplished by the comparative threshold cycle (C_T) method using the Bio-Rad (Hercules, CA) MyiQ real-time PCR (RT-PCR) detection system with fluorescein-spiked SYBR green as the fluorophore. Binding of the dye to the DNA leads to an increase in fluorescence and is the basis for the detection of the specific cDNA PCR product. The cycle at which the fluorescence intensity generated by a specific PCR product passes a fixed threshold is defined as the C_T. The primers for RT-PCR analysis were designed using Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). The lengths of the primers were 23 or 24 nucleotides, the melting temperature was 60°C, and they amplified 100- to 110-bp specific fragments. Efficiency of amplification was confirmed by analyzing melting curves of each amplicon following the amplification with template dilution over four orders of magnitude. The efficiency of amplification was between 97.6 and 111.7% for each primer pair, showing that the amplicons were generated with similar efficiency. Primers used in this study are listed in Table S12 in the supplemental material.

All RT-PCRs were performed in 1× SYBR green master mix (Bio-Rad) with specific primers (2 ng/μl) and 0.4 ng/μl of cDNA sample in a 25-μl volume. The RT-PCR protocol included one cycle at 95°C for 90 seconds, followed by 40 cycles with two steps each at 95°C for 15 seconds and at 60°C for 1 min. RT-PCR amplification with primers to the *gyrA* gene was used for normalization. Nontemplate controls were included to confirm the absence of primer-dimer formation. All samples, including nontemplate controls, were run in triplicates. The cDNA samples used as templates for RT-PCRs were identical to those used in microarray experiments.

The comparison of gene expression in various conditions was accomplished by comparison of the C_T values that were automatically generated by the MyiQ software. The relative comparison of expression of the gene of interest (goi) in two different conditions (goi1 and goi2) was presented as fold change (FC). All FC values of the genes of interest were normalized to *gyrA*, since it produced little variation of expression. Previously published mathematical models (19, 23) for relative quantifications of RT-PCR data were performed. Briefly, the ΔC_T was calculated by subtracting the *gyrA* C_T values from the goi C_T values. Calculation of $\Delta\Delta C_T$ involved subtraction of the ΔC_T goi2 value from the ΔC_T goi1 value. The FC value was calculated using formula FC = $2^{-\Delta\Delta C_T}$. Correlation analysis of RT-PCR and microarray data sets was performed using Excel.

Microarray accession numbers. Microarray data are available at the National Center for Biotechnology Information Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo) under platform and series accession numbers GPL4769 and GSE6973, respectively.

RESULTS

Previous computational analyses of the *S. mutans* UA159 genome database showed the presence of 14 PTS systems and

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4 ABC transporters for probable transport of sugars and sugar alcohols (4). Additional analysis confirmed the presence of three carbohydrate ABC transporters, with one of them (SMU.827 and -828) possibly being a carbohydrate exporter involved in cell-wall biogenesis (41). The fourth ABC transporter (SMU.1118 to -1120) was proven to be responsible for ribonucleoside uptake (39). Consequently, the latter two ABC transporters were not analyzed in this study.

Global transcriptional patterns were obtained following growth on different carbohydrates, including monosaccharides (glucose, mannose, fructose, and galactose), disaccharides (sucrose, lactose, maltose, and trehalose), a β-glucoside (cellobiose), oligosaccharides (raffinose, stachyose, and maltotriose), and a sugar-alcohol (mannitol). To ensure reproducibility of the data, the cultures were always grown to a similar optical density. We have also observed that the growth phase of the cultures had a profound effect on the data obtained from pairwise comparison. Because of this, a growth curve of UA159 was generated for each condition tested (see Fig. S1 in the supplemental material), and the samples that exhibited similar growth were compared (see Materials and Methods). Additionally, all samples were also compared to glucose-grown cultures.

A total of 48 samples (10 biological replicates each for glucose and fructose and 2 to 4 replicates for every other tested sugar) were prepared. The microarray results are presented in Tables S1 to S11 in the supplemental material. The results generated for each biological replicate of each condition were compared to the results for glucose-grown samples or another relevant condition, and several tables were generated for each trial and for each of the studied sugars. All tables representing results for each condition were compared, and consistent changes in gene expression were compiled. For example, pairwise comparisons of two glucose- and two fructose-grown samples showed differential expression in 266, 337, 376, and 401 genes when all possible combinations were performed. Among these genes, 22 showed more than a twofold decrease and 46 genes showed a twofold or greater increase in transcription. However, all four paired treatment conditions revealed 13 common genes with decreased and 10 genes with increased transcription (highlighted in Table S1 in the supplemental material) in glucose compared to fructose. Similar comparisons were performed for all conditions (see Tables S2 to S11 in the supplemental material).

In the presence of fructose, one operon (SMU.1961, mannose/fructose-specific EIIA; SMU.1960, mannose/fructose-specific EIIB; SMU.1958, mannose/fructose-specific EIIC; SMU.1957, mannose/fructose specific EIID; and SMU.1956, part of the mannose/fructose-specific PTS) (Fig. 1), encoding a putative EII, showed dramatically increased transcription, with an induction level of 5- to 11-fold for different genes of the operon (Fig. 2B and Fig. 3A; see Table S1 in the supplemental material), suggesting that this is an inducible fructose EII. Microarray results showed an 11-fold-increased transcription for open reading frame (ORF) SMU.1956 in the presence of fructose, suggesting that this gene, initially annotated as a hypothetical protein, is an integral part of the operon and probably part of EII for the inducible fructose PTS.

The operon characterized in this study as an inducible fructose operon (SMU.1956 to -1961) (Fig. 1) was also differentially transcribed in the presence of mannose. The genes of this operon were induced 14- to 37-fold in the presence of mannose compared to mannitol-grown cells (Fig. 3A; see Table S2 in the supplemental material). Therefore, this EII is an inducible fructose and mannose EII, which we named $EII^{Fru/Man}$. This is the first study that demonstrates the existence of an inducible mannose transporter.

When strain UA159 was grown in the presence of sucrose and dextranase, genes for three sugar transporters exhibited differential expression; i.e., the genes for a maltose/maltodextrin ABC transporter (SMU.1568 to -1571) (Fig. 1) showed a two- to threefold decrease, the genes for a raffinose ABC transporter (SMU.878 to -882) (Fig. 1) also exhibited a two- to threefold decrease, and the genes for an inducible $EII^{Fru/Man}$ (Fig. 1) showed an approximate twofold increase in transcription compared to those in the glucose- and dextranase-grown cells (Fig. 3A; see Table S3 in the supplemental material).

In the presence of lactose, the transcription of two transporter genes, SMU.1491 (EIIBC) and SMU.1492 (EIIA) (Fig. 1), was increased 15- and 24-fold, respectively (Fig. 3B; see Table S4 in the supplemental material). These two genes are an integral part of the lactose-tagatose operon (14–16, 27), and they are also annotated as a putative EII^{Lac}; therefore, we conclude that this is a major lactose transporter. All genes for the lactose-tagatose 6-phosphate and Leloir pathway were also induced (see Table S4 in the supplemental material). Additionally, ORFs SMU.1488 and SMU.1489 were highly induced in lactose-grown cells (see Table S4 in the supplemental material). These two genes are located next to the gene for phospho-β-galactosidase, the last gene of the lactose operon. Their elevated transcription in lactose suggests that they are also involved in lactose and/or galactose metabolism. The same genes that were induced in lactose also exhibited differential transcription in galactose-grown cells (Fig. 3B; see Table S5 in the supplemental material).

Three of the *S. mutans* transporters were differentially expressed in raffinose-grown cells. Genes encoding an ABC transporter (SMU.878 to -880 and SMU.882) (Fig. 1), which are a part of the MSM operon, showed a 2.5- to 3.5-fold increase in transcription, whereas transcription of the genes for $EII^{Fru/Man}$ (Fig. 1) was increased 4- to 11-fold (Fig. 3B; see Table S6 in the supplemental material). Interestingly, transcription of the genes for EII^{Lac} (Fig. 1) was elevated more than six- and ninefold, respectively (Fig. 3B; see Table S6 in the supplemental material). In addition to differential transcription of the genes for transport, raffinose-grown cells exhibited differential transcription in several metabolic operons: lactose-tagatose, galactose, and MSM (see Table S6 in the supplemental material).

The same transporters and catabolic operons induced following growth in raffinose were also induced in stachyose-grown cells (Fig. 3B; see Table S7 in the supplemental material). This result was not surprising because stachyose is an oligosaccharide very similar to raffinose and the only difference between the two is an additional galactose moiety in stachyose.

In the presence of trehalose, two genes of the putative trehalose operon were differentially transcribed compared to other conditions. The gene for putative EII^{Tre} (SMU.2038) was found to have more than a 22-fold increase in transcription (Fig. 3C; see Table S8 in the supplemental material). Also,

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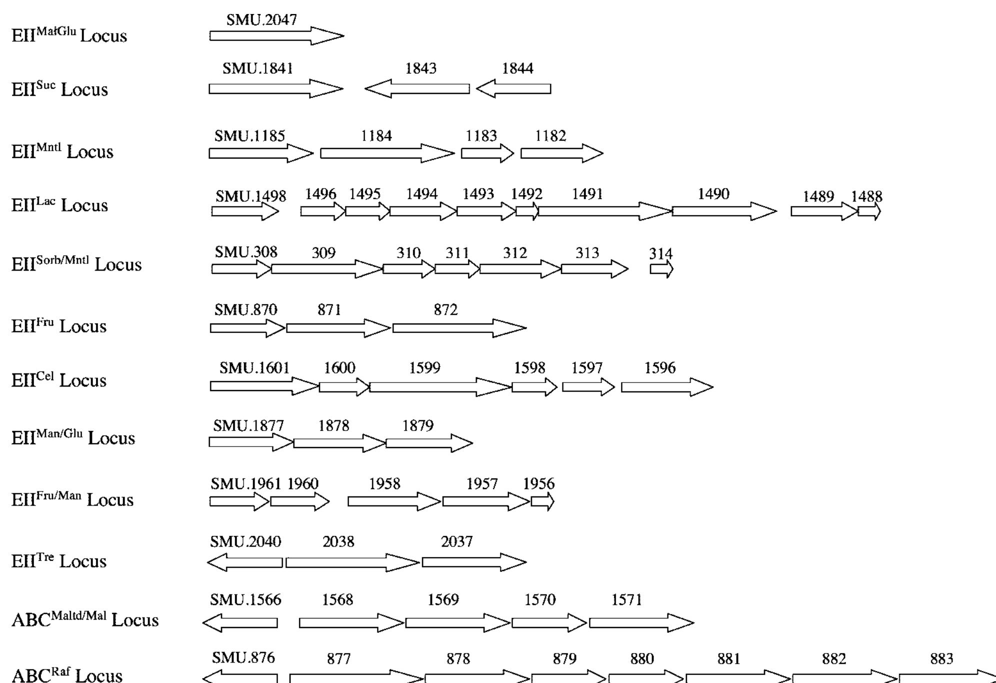

FIG. 1. Schematic presentation of the genetic loci involved in transport and catabolism of the sugars analyzed in this study. Arrows represent the genes. The probable function of each gene is listed below. The numbers represent numeric designation of the ORFs listed in the *S. mutans* UA159 sequencing database (NCBI) (4). The ORFs are listed in 5′-to-3′ order. Descriptions of the gene products are redefined compared to the original annotation (4) as a result of this transcriptional study. The probable functions of the genes are as follows: SMU.2047, PTS system, glucose/maltose-specific IIABC; SMU.1841, PTS system, sucrose-specific IIABC; SMU.1843, sucrose-6-phosphate hydrolase; SMU.1844, sucrose operon repressor; SMU.1185, PTS system, mannitol-specific EIIBC; SMU.1184, transcriptional regulator, antiterminator; SMU.1183, PTS system, mannitol-specific EIIA; SMU.1182, mannitol-1-phosphate dehydrogenase; SMU.1498, lactose operon repressor; SMU.1496, galactose-6-phosphate isomerase, subunit LacA; SMU.1495, galactose-6-phosphate isomerase, subunit LacB; SMU.1494, tagatose-6-phosphate kinase; SMU.1493, tagatose-1,6-bisphosphate aldolase; SMU.1492, PTS system, lactose-specific EIIA (permease EIIA-LAC); SMU.1491, PTS system, lactose-specific EIIBC (permease EIIBC-LAC); SMU.1490, 6-phospho-beta-galactosidase; SMU.1489, part of lactose regulon, LacX; SMU.1488, part of lactose regulon; SMU.308, sorbitol and mannitol-6-phosphate dehydrogenase; SMU.309, regulator of sorbitol and mannitol operon; SMU.310, sorbitol and mannitol operon activator; SMU.311, PTS system, sorbitol- and mannitol-specific EIIC2; SMU.312; PTS system, sorbitol- and mannitol-specific EIIBC; SMU.313, PTS system, sorbitol- and mannitol-specific EIIA; SMU.314, part of sorbitol and mannitol regulon; SMU.870, transcriptional regulator of fructose operon; SMU.871, fructose-phosphate kinase; SMU.872, PTS system, fructose-specific EIIABC; SMU.1601, phospho-beta-glucosidase; SMU.1600, PTS system, cellobiose-specific EIIB; SMU.1599, transcriptional regulator of cellobiose operon, possible antiterminator; SMU.1598, PTS system, cellobiose-specific EIIA; SMU.1597, part of cellobiose operon; SMU.1596, PTS system, cellobiose-specific EIIC; SMU.1877, PTS system, mannose/glucose-specific EIIAB; SMU.1878, PTS system, mannose/glucose-specific EIIC; SMU.1879, PTS system, mannose/glucose-specific EIID; SMU.1961, PTS system, mannose/fructose-specific EIIA; SMU.1960, PTS system, mannose/fructose-specific EIIB; SMU.1958, PTS system, mannose/fructose-specific EIIC; SMU.1957, PTS system, mannose/fructose-specific EIID; SMU.1956, part of the mannose/fructose-specific PTS; SMU.2040, transcriptional regulator, repressor of the trehalose operon; SMU.2038, PTS system, trehalose-specific EIIABC; SMU.2037, trehalose-6-phosphate hydrolase TreA; SMU.1566, maltodextrin and maltose operon transcriptional repressor; SMU.1568, maltodextrin and maltose ABC transporter, sugar-binding protein MalX; SMU.1569, maltodextrin and maltose ABC transporter, MalG permease; SMU.1570, maltodextrin and maltose ABC transporter, permease; SMU.1571, ABC transporter, ATP-binding protein; SMU.876, MSM operon regulatory protein; SMU.877, MSM operon, alpha-galactosidase; SMU.878, MSM ABC transporter, sugar-binding protein precursor MsmE; SMU.879, MSM ABC transporter, permease protein MsmF; SMU.880, MSM ABC transporter, permease protein MsmG; SMU.881, MSM operon, sucrose phosphorylase, GtfA; SMU.882, MSM operon, ABC transporter, ATP-binding protein MsmK; SMU.883, MSM operon, dextran glucosidase DexB.

transcription of the gene for a putative trehalose-6-phosphate hydrolase (SMU.2037) was increased more than 18-fold.

Transcription of the operon for an ABC transporter (SMU.1568 to -1571 [sugar-binding protein, two permease components, and ATP-binding protein, respectively]) (Fig. 1) was fivefold increased in the presence of maltose (see Table S9 in the supplemental material) and fourfold increased in the presence of maltotriose (see Table S10 in the supplemental material), suggesting its involvement in maltose and maltotriose transport (Fig. 3C).

The genes of the EII^{Cel} locus (SMU.1596 to -1601) (Fig. 1) were differentially transcribed, with the induction level being 11- to 42-fold higher for different genes of the same operon over its basal level of transcription (Fig. 3C; see Table S11 in the supplemental material). The gene SMU.1597, originally annotated as encoding a "conserved hypothetical protein," was induced 52-fold. Further analysis of its deduced amino acid sequence demonstrated membrane protein characteristics (data not shown), suggesting its involvement in cellobiose transport.

In the presence of mannitol, 12 genes distributed in two loci were differentially transcribed. The first locus included seven genes (SMU.308, sorbitol/mannitol-6-phosphate dehydrogenase; SMU.309, regulator of sorbitol/mannitol operon; SMU.310, sorbitol/mannitol operon activator; SMU.311, PTS sorbitol/mannitol-specific EIIC2; SMU.312; PTS sorbitol/mannitol-specific EIIBC; SMU.313, PTS sorbitol/mannitol-specific EIIA; and

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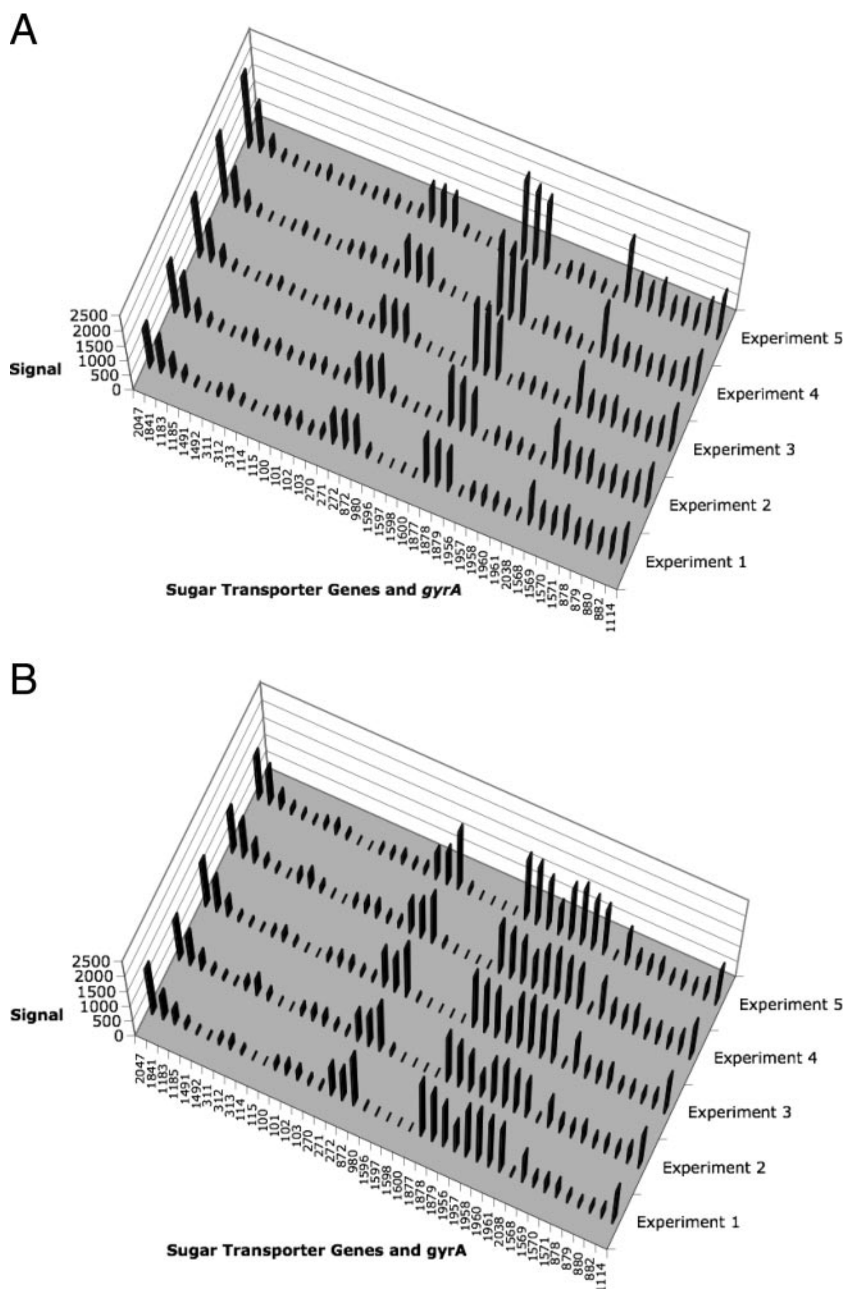

FIG. 2. Differential transcription of the genes for PTS EII and ABC transporters following growth of *S. mutans* UA159 in glucose and fructose. Microarray results are presented as vertical bars. The numerical ORF designations for genes encoding sugar transporters are presented on the *x* axis. A full list of the gene product names is presented in Fig. 1. The value of the normalized expression signal is presented on the *y* axis. Transcription of the gene *gyrA* (SMU.1114) served as an endogenous control because it did not show significant variation of transcription under the conditions compared. A total of 10 glucose- and 10 fructose-grown samples were analyzed in five independent experiments. Two biological replicates were grown and tested in each experiment for each sugar, and thus we present the average results for two samples grown under the same condition: A, glucose; B, fructose.

SMU.314, part of sorbitol/mannitol regulon) and was reannotated as the sorbitol/mannitol operon (Fig. 1). The induction levels of the different genes of the sorbitol/mannitol operon varied between 10- and 16-fold (Fig. 3C; see Tables S2 and S11 in the supplemental material). The second locus was a well-characterized mannitol operon (SMU.1185, PTS mannitol-specific EIIBC; SMU.1184, transcriptional regulator; SMU.1183, PTS mannitol-specific EIIA; and SMU.1182, mannitol-1-phosphate dehydrogenase) (Fig. 1). Transcription of the different genes of this operon was between 4- and 10-fold higher in mannitol compared to cellobiose (Fig. 3C; see Tables S2 and S11 in the supplemental material).

Transcription of selected sugar transporter genes in different conditions was also tested using RT-PCR. Specific genes for sugar transport (for ABC transporters, the gene for the solute-binding receptor; for PTS, one of the genes for EII) were

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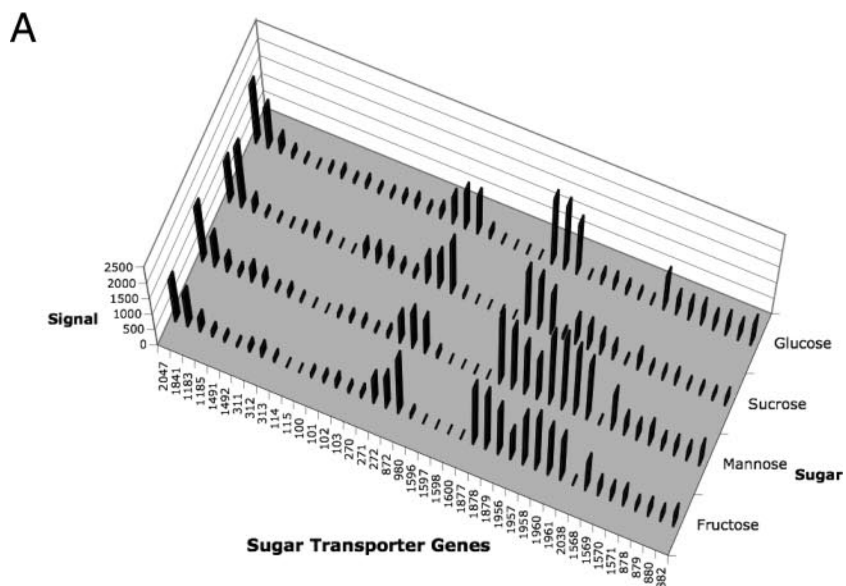

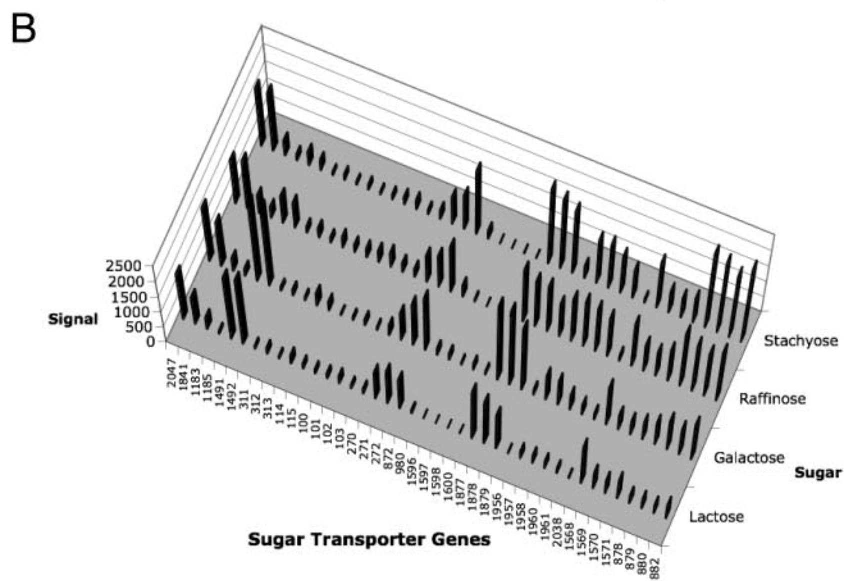

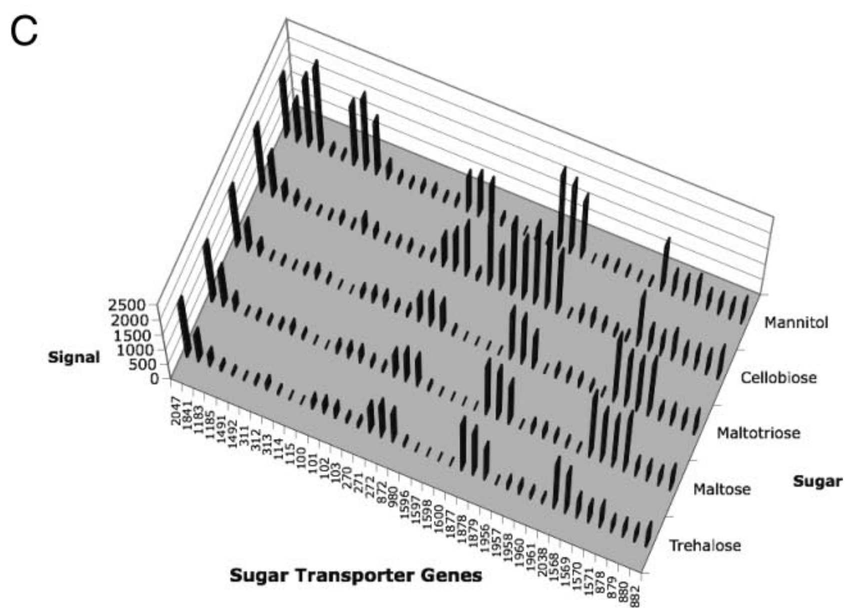

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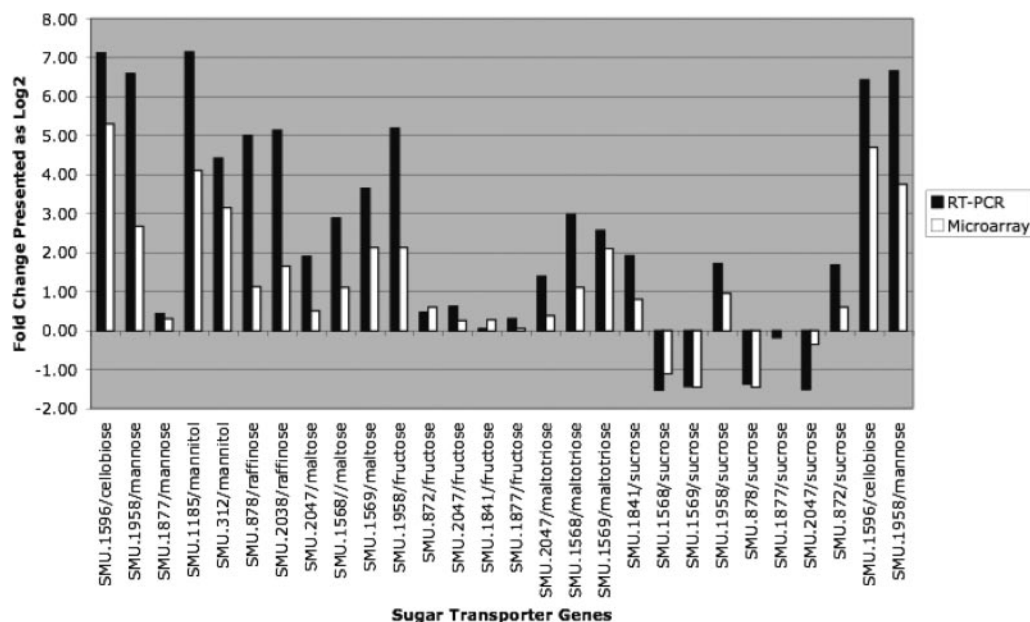

FIG. 4. Comparison of microarray and RT-PCR results for selected sugar transporter genes. Vertical bars represent the FC of gene transcription for an experimental condition versus baseline. In most cases the baseline was a glucose-grown sample, except for samples grown in cellobiose and mannose, which were also compared to mannitol. For both sets of results, the FCs are presented in $\log_2$ scale (*y* axis). Results presented above the *x* axis represent an increase in transcription, and results below the *x* axis represent a decrease in transcription. The numerical ORF designations for the sugar transporter genes are presented on the *x* axis. A full list of gene product names is presented in Fig. 1. Experimental conditions (left to right) compared to glucose-grown samples: SMU.1596, cellobiose; SMU.1958 and SMU.1877, mannose; SMU.1185 and SMU.312, mannitol; SMU.878 and SMU.2038, raffinose; SMU.2047, SMU.1568, and SMU.1569, maltose; SMU.1958, SMU.872, SMU.2047, SMU.1841, and SMU.1877, fructose; SMU.2047, SMU.1568, and SMU.1569, maltotriose; SMU.1841, SMU.1568, SMU.1569, SMU.1958, SMU.878, SMU.1877, SMU.2047, and SMU.872, sucrose. Experimental conditions compared to mannitol-grown samples: SMU.1596, cellobiose; SMU.1958, mannose.

selected for this analysis. The microarray and RT-PCR results were compared, and although the RT-PCR results revealed a higher fold change range than the microarray data, these two data sets showed a correlation coefficient of 0.92 for data presented on a $\log_2$ scale (Fig. 4). The RT-PCR data revealed a 3.8-fold ($\log_2 = 1.9$) increase in transcription of *scrA*, a 3.2-fold ($\log_2 = 1.7$) increase in transcription of SMU.872 (encoding EII^{Fru}), and a 2.8-fold ($\log_2 = 1.5$) decrease in transcription of SMU.2047 (encoding $EII^{Mal/Glu}$) in the presence of sucrose (Fig. 4). RT-PCR data also revealed a 3.7-fold ($\log_2 = 1.9$) and 2.6-fold ($\log_2 = 1.4$) elevation in transcription of one PTS (SMU.2047) in the presence of maltose and maltotriose, respectively (Fig. 4).

DISCUSSION

Global differential transcription profiles were obtained for growth of *S. mutans* in 13 different sugars using microarrays. The results achieved for all samples grown in the same sugar showed excellent reproducibility, as presented in Fig. 1 for glucose- and fructose-grown samples. Nevertheless, selected microarray results were verified using RT-PCR, and these two data sets showed an excellent correlation (Fig. 4).

Microarray results indicated that fructose was transported by the PTS in *S. mutans*. In fact, previous studies indicated that fructose was transported into the cell via an inducible PTS and a constitutive PTS (11, 40). Wen and colleagues (40) have characterized transcription of both transporters. Comparison of both published gene clusters and their flanking regions to the genome database (4) revealed that the EIIs for these PTSs were designated as SMU.872 (EII^{Fru}, constitutive) and SMU.113 to -116 (inducible). The microarray results confirmed that SMU.872 was constitutively expressed (Fig. 2B and 3A). However, our previous findings (V. Pham, G. Savic, N. McElwee, and D. Ajdic, presented at the Streptococcal Genetics meeting, Saint-Malo, France, 18 to 21 June, 2006) and current transcriptional analysis revealed a different fructose-induced operon (SMU.1956 to -1961) (Fig. 2B and 3A). Recently published data (42) concerning fructose metabolism confirmed our results. The previously reported fructose inducible operon (SMU.113 to -116) (40) showed very low transcrip-

FIG. 3. Differential transcription of genes for PTS EII and ABC transporters following growth of *S. mutans* UA159 in different sugars. Microarray results are presented as vertical bars. The numerical ORF designations for genes encoding sugar transporters are presented on the *x* axis. A full list of the gene product names is presented in Fig. 1. The value of the normalized expression signal is presented on the *y* axis. Two biological replicates were analyzed for each sugar, and thus we present the average results for samples grown under each condition. A, fructose, mannose, sucrose with dextranase, and glucose with dextranase; B, lactose, galactose, raffinose, and stachyose; C, trehalose, maltose, maltotriose, cellobiose, and mannitol.

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tion in fructose-grown cells. Furthermore, this operon exhibited low expression in every condition tested in this study. It is not completely clear why this operon was not transcribed in the presence of fructose. One explanation could be that there is a mutation in the promoter region that hinders operon transcription in the tested strain. Alternatively, this may be not a fructose EII but an EII for some unknown sugar.

Growth in sucrose is inherently difficult due to dextran (glucan) production. Strain UA159 formed visible aggregates when grown in the presence of sucrose, and consequently it was difficult to obtain an accurate optical density of the culture. Since the growth rate of the culture and the number of the cells in each sample compared were critical for microarrays, we could not perform microarray experiments following growth in sucrose. To address this question, we have analyzed sucrose transport in UA159 grown in a culture supplemented with extracellular endodextranase. This enzyme catalyzes the endohydrolysis at the α-1,6-glycosidic bond of dextran (glucan) and releases short isomaltosaccharides consisting of three to five glucose molecules. *S. mutans* is also known to produce an extracellular endodextranase that cleaves dextran (12). The activity of this enzyme is apparently well synchronized with the production of other enzymes involved in extracellular sugar metabolism, resulting in the accumulation of dextrans if sucrose is available. However, when the cells were grown in the presence of a high concentration of dextranase, dextran was cleaved and aggregation of *S. mutans* was prevented.

Induction of $EII^{Fru/Man}$ following growth in sucrose was expected because sucrose serves as a substrate for fructosyltransferase (13) and fructanase (8, 38). These extracellular enzymes release the fructose moiety from sucrose, which is then taken up by its specific PTS. Although fructose is the most probable substrate for this PTS, we cannot rule out the possibility that this PTS also transports sucrose.

It is not completely clear why there was decreased transcription of the maltodextrin ABC transporter (SMU.1568 to -1571) in the presence of sucrose and dextranase, since one would expect that this condition would favor production of isomaltosaccharides. The high concentration of dextranase added to the cultures possibly lowered the concentration of longer isomaltosaccharides (maltodextrins). Therefore, longer isomaltosaccharides might be a substrate for this ABC transporter, and their lower concentration in the culture might result in its decreased transcription. Alternatively, since the control samples (glucose- and dextranase-grown cultures) did not contain products of hydrolyzed glucans, it is possible that this transporter exhibits some specificity for glucose.

It was surprising to find a decreased transcription of the raffinose ABC transporter (SMU.878 to -882) upon growth in sucrose given that it was previously reported that this transporter takes up multiple sugars, including sucrose, in strain LT11 (17, 28, 34). Our microarray data demonstrated that the transcription of the raffinose transporter is very low in sucrose compared to any other sugar tested (Fig. 3). This result suggested that neither the raffinose nor maltodextrin ABC transporter was involved in sucrose transport under the conditions tested. Thus, sucrose is not an inducer of the two ABC operons. However, it is possible that upon their induction by the specific substrates, these transporters may play a minor role in sucrose transport.

Several transporters in *S. mutans* take up sucrose, and among them is the best-characterized, high-affinity sucrose PTS. The gene for EII^{Suc} is located in an operon that also includes the gene for sucrose-6-phosphate hydrolase (30). Therefore, sucrose is converted to sucrose-6-phosphate during transport into the cell and is subsequently hydrolyzed to glucose-6-phosphate and fructose. A BLAST search of the nucleotide sequence for the EII^{Suc} gene (*scrA*) revealed that this gene was designated SMU.1841 in the genome database (4). The *scrA* gene was consistently highly transcribed under every condition tested in this study. However, the RT-PCR data revealed increased transcription of *scrA* in the presence of sucrose (Fig. 4), suggesting that even highly expressed EII^{Scr} could be further induced. RT-PCR data also revealed increased transcription of SMU.872 (encoding EII^{Fru}) and decreased transcription of SMU.2047 (encoding $EII^{Mal/Glu}$) in the presence of sucrose. EII^{Fru} is a part of the PTS that has been characterized as a constitutive fructose transporter (11, 40), so it was not surprising to find further increase of transcription of the EII^{Fru} gene in sucrose, since the fructose moiety of this disaccharide can be released extracellularly. Alternatively, the possibility exists that EII^{Fru} might transport sucrose. It was intriguing to find a slight transcriptional decrease of the $EII^{Mal/Glu}$ gene. This PTS could also be involved in maltodextrin transport, and its lower transcription might be due to a decreased concentration of longer isomaltosaccharides in the culture grown with dextranase.

Several studies have suggested the existence of a second inducible sucrose PTS (25, 32). We did not detect the induction of any PTS, except for those for fructose uptake, following *S. mutans* growth in sucrose. It has been suggested that the second PTS for sucrose was the trehalose PTS in *S. mutans* GS5 (25). We did not detect induction of the trehalose PTS in the presence of sucrose. Furthermore, EII^{Tre} exhibited minimal activity under any growth condition except when trehalose was present. Therefore, this PTS cannot be induced by sucrose (or any other sugar tested) in UA159. However, it is possible that this PTS exhibits some affinity for sucrose after it is induced by trehalose. Alternatively, the results presented here may be strain specific, since it has been known that there are some strain-specific variations in sugar transport (37).

Similar to sucrose, maltose serves as a primer for the synthesis of water-soluble dextrans (10). These molecules are cleaved by dextranase to isomaltosaccharides that can be taken up by *S. mutans*. Therefore, the maltose/maltotriose ABC transporter (SMU.1568 to -1571) may also be responsible for transport of longer isomaltosaccharides. RT-PCR data revealed elevated transcription of one PTS (SMU.2047) in the presence of maltose and maltotriose (Fig. 4). This transporter has been previously characterized as a glucose transporter (1, 36). Our transcriptional analysis also suggests its involvement in maltose and maltotriose uptake.

As mentioned earlier, in addition to EII^{Lac} genes, all genes for the lactose-tagatose 6-phosphate pathway were also induced in the presence of lactose. This was an expected result because following lactose uptake, lactose-6-phosphate is cleaved by phospho-β-galactosidase (encoded by the last gene of the operon) to galactose-6-phosphate and glucose (15). Galactose-6-phosphate is then metabolized by the tagatose-6-phosphate pathway. Interestingly, the galactose operon was also induced,

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suggesting that the galactose moiety of lactose is metabolized simultaneously through the Leloir and tagatose-6-phosphate pathways. The Leloir pathway seems to be the primary pathway for galactose catabolism when *S. mutans* grows on galactose as a sole carbon source. The first enzyme of the Leloir pathway, galactokinase, catalyzes the phosphorylation of galactose to galactose-1-phosphate. Inactivation of the galactokinase gene completely abolished the growth of *S. mutans* in galactose (3, 5). Therefore, the primary galactose uptake might not be mediated by a PTS. However, when galactokinase is intact, both the Leloir and the tagatose-6-phosphate pathways are active following growth in galactose (3). Our microarray data confirmed this result (see Table S5 in the supplemental material). Therefore, *S. mutans* might possess an enzyme that converts some intermediate of the Leloir pathway into one of the intermediates of the tagatose-6-phosphate pathway (and vice versa). Interestingly, transcription of the EII^{Lac} genes was fully induced in galactose, suggesting that this PTS might also transport galactose. If this were true, galactose would be phosphorylated during PTS transport and utilized through the tagatose-6-phosphate pathway. However, strain UA159 cannot grow on galactose if galactokinase is inactivated. Therefore, the lactose PTS might not be a primary transporter for galactose. Although the microarray data (see Table S5 in the supplemental material) clearly showed induction of lactose, tagatose-6-phosphate, and galactose operons following growth in galactose, we could not with confidence detect a non-PTS transporter dedicated to galactose transport. This was most likely due to a different growth rate of UA159 in galactose compared to other sugars, in which a large number of genes exhibited differential transcription.

Russell and colleagues have shown that the MSM transporter was responsible for uptake and metabolism of melibiose, raffinose, and isomaltotriose and for the metabolism of sucrose (28, 34). Our results confirmed that the ABC transporter of the MSM operon is the major transporter for raffinose uptake. Induction of the fructose transporter (Fig. 3B) clearly shows the presence of a fructose moiety released from raffinose by extracellular enzymes, presumably fructan hydrolase (fructanase, exo-β-D-fructosidase) (6, 8, 38) and fructosyltransferase (13). These enzymes are capable of cleaving the fructose moiety from raffinose, which is then efficiently taken up by the cell and utilized as an energy source. The lactose transporter might also be able to take up raffinose or the disaccharide known as melibiose that is left following the release of fructose. Melibiose is cleaved by intracellular alpha-galactosidase to galactose and glucose, and these monosaccharides are utilized as an energy source (28). The microarray data clearly show that all three monosaccharides of raffinose and stachyose (galactose, glucose, and fructose) are utilized by *S. mutans* during the mid-log phase of growth and that galactose is metabolized simultaneously through the Leloir and tagatose-6-phosphate pathways.

In the presence of trehalose, transcription of an operon encoding putative EII^{Tre} (SMU.2038) and trehalose-6-phosphate hydrolase (SMU.2037) was increased. The microarray result confirmed that this operon indeed encodes a major trehalose-inducible EII^{Tre} and accompanied hydrolase. Presumably, the PTS takes up and phosphorylates trehalose to trehalose-6-phosphate that is then hydrolyzed to glucose and glucose-6-phosphate. Although the presence of an inducible trehalose PTS was demonstrated previously (25), this is the first study that characterizes the trehalose operon and its transcription.

S. mutans is capable of utilizing β-glucosides such as cellobiose, esculin, arbutin, and salicin. Metabolic and transport genes for these sugars are organized in three loci. Cellobiose and salicin are utilized by proteins encoded in the *cel* locus (SMU.1596 to -1601) (22). The second locus, designated *bgl* (SMU.977 to -985), consists of the genes for PTS transport (EII, SMU.980) and the metabolism of esculin (7, 22). Arbutin is hydrolyzed by its own phospho-β-glucosidase (encoded by the *arb* gene, SMU.1102), but it is transported by the *cel* or *bgl* system (22). Although the microarray data showed that transcription of EII^{Cel} (SMU.1596, SMU.1598, and SMU.1600) varied severalfold in different sugars, the signal was always very low, suggesting that this operon is substrate inducible and there was only a basal level of transcription under all previously tested conditions (Fig. 3). To test this hypothesis, a microarray analysis was performed following growth in cellobiose. As expected, the genes for EII^{Cel}, the accompanying phospho-β-glucosidase (SMU.1601), and the regulator (SMU.1599) were differentially transcribed, with an induction level much higher than the basal level of transcription (Fig. 3C; see Table S11 in the supplemental material). We conclude that cellobiose is transported by an inducible PTS and subsequently hydrolyzed by phospho-β-glucosidase, CelA.

Previous studies have demonstrated that *S. mutans* possessed two independent inducible PTSs for transport of mannitol and sorbitol (20). The accompanying catabolic enzymes, mannitol-1-phosphate and sorbitol-6-phosphate dehydrogenases that convert the respective phosphorylated sugars to fructose-6-phosphate, were also inducible (20). However, our results showed that mannitol induced both mannitol and sorbitol operons, suggesting that both operons were utilized for transport and metabolism of mannitol in UA159. Consequently, sorbitol EII was renamed $EII^{Sorb/Mntl}$.

Analysis of global transcription profiles did not detect significant induction of any sugar transporter in the presence of glucose compared to any other condition. However, genes for five EIIs were consistently highly transcribed, and two ABC transporters were moderately expressed, whereas the other transporters showed very low transcription in glucose (Fig. 2A and 3A; see Tables S1 and S3 to S10 in the supplemental material). The presence of a glucose PTS in *S. mutans* was reported decades ago (9, 18, 31). In fact, *S. mutans* harbors at least two glucose PTS transporters (21, 36). One of them has been characterized and demonstrated to take up mannose, glucose, and 2-deoxyglucose (2, 36). Another is responsible for glucose, 2-deoxyglucose, and α-methylglucoside transport (1, 36). Comparison of the two glucose PTSs to the genome database (4) showed that they were designated SMU.1877 to -1879 (SMU.1877, mannose/glucose-specific EIIAB; SMU.1878, mannose/glucose-specific EIIC; SMU.1879, mannose/glucose-specific EIID) and SMU.2047 (glucose/maltose-specific EIIABC), respectively (Fig. 1). RT-PCR data revealed that the second glucose PTS was also a maltose transporter. Both glucose transporters were consistently highly transcribed in UA159. Additionally, both ABC sugar transporters ABC^{Raf} and $ABC^{Maltd/Mal}$ showed differential transcription in glucose,

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suggesting that they might have some affinity for this carbohydrate.

The five PTS transporters that were highly transcribed in glucose showed a similar level of transcription in all other sugars used in this study (Fig. 2 and 3). It is not completely clear which carbohydrates are substrates for these transporters. As mentioned earlier, $EII^{Mal/Glu}$ is specific for maltose and glucose, and $EII^{Man/Glu}$ is specific for mannose and glucose. Sato and colleagues have cloned and sequenced a sucrose-specific transporter of *S. mutans* (30). Comparison of this sequence to the genome database showed that one of the five highly expressed EIIs is EII^{Suc}. In addition to the inducible fructose PTS, *S. mutans* also possesses a constitutive fructose PTS (11, 40). Comparison of its sequence to the genome database revealed that EII^{Fru} was also one of the five highly transcribed transporters. The specificity of the fifth highly transcribed EII (SMU.270 to -272) remains unknown.

Although the five PTSs were highly transcribed under every condition tested in this study, some showed transcription that was further elevated if the particular sugar was present. The gene for high-affinity EII^{Suc}, previously characterized as being constitutive, showed a further increase in transcription in sucrose compared to glucose (Fig. 4). Similarly, RT-PCR results revealed that the transcription of the genes for $EII^{Mal/Glu}$ and EII^{Fru} was elevated severalfold in the presence of maltose and sucrose, respectively (Fig. 4). These data confirm that the transcription of the "constitutive" EII genes can be further increased under the appropriate environmental conditions.

Evidence that *S. mutans* constantly expresses five PTSs suggests that their substrates, glucose, fructose, maltose, and sucrose, might be preferable sugars for this organism. These are also the main dietary sugars, and therefore this bacterium is capable of their immediate utilization as they become available. It is obvious that these transporters are important for *S. mutans*, since the cell expresses them continuously. In addition to their role in instant sugar uptake, it is also possible that these PTSs are involved in the regulation of sugar transport and in catabolite repression. Experiments conducted with $EII^{Man/Glu}$ mutants indicated that preferential utilization of glucose over lactose depended on the presence of this EII (36).

Transcription of the genes for PTS EI and Hpr was consistently high following growth in different carbohydrates, suggesting that these genes were not regulated by a particular sugar used in this study. $EII^{\beta\text{-}Glu}$ (SMU.980) was not induced by any sugar used in this study, but it has been previously suggested that it transported a β-glucoside (7, 22). Two EIIs (SMU.114 and -115 and SMU.100 to -103) were repressed under every condition tested in this study, and therefore their sugar specificity remains unknown.

Conclusions. Analysis of the global transcription patterns following growth on 13 different carbohydrates revealed two types of sugar transporters in *S. mutans*. Members of the PTS family, the primary carbohydrate transporters of gram-positive bacteria, transported most of the sugars. In fact, monosaccharides, disaccharides, β-glucosides, and sugar alcohols were all transported by PTSs. In contrast, the ABC transporters appear to be specialized for transport of oligosaccharides in *S. mutans*.

Differential expression profiles not only identified the sugar transporters but also confirmed the presence of the previously studied genes involved in the catabolism of sugars and allowed identification of new genes and operons. The microarray results, in conjunction with genome sequencing and annotation, provided information about the exact locations and compositions of the operons involved in sugar transport and metabolism. In nine operons, transporter genes for EII are an integral part of operons consisting of genes encoding enzymes necessary for the hydrolysis and catabolism of the transported sugar. Colocalization of the genes for sugar transport and catabolism indicates their coregulation. Transcription of the majority of the sugar transporters and accompanied catabolic genes was induced by their specific substrate. The common feature of all of these operons was the presence of a regulatory gene that was presumably responsible for their transcriptional regulation.

Although most of the sugars tested in this study specifically induced genes for their own transport and catabolism, five of the PTSs were consistently highly expressed regardless of the sugar source. These transcription profiles were consistent with the published functional studies for glucose/mannose and fructose PTSs. Microarray data provided evidence that the maltose/glucose PTS and the sucrose PTS were also consistently highly expressed, suggesting that glucose, fructose, maltose, and sucrose might be the preferred sugars for *S. mutans*. Additionally, these transporters might be involved in the regulation of sugar transport and metabolism, as shown for $EII^{Man/Glu}$ (1, 2, 36).

All inducible PTSs showed very low transcription in the absence of the specific sugar substrate, suggesting their high specificity for particular sugars. The ABC transporters showed low to moderate transcription in different sugars, suggesting that they might transport multiple substrates. Interestingly, the transcription of these ABC transporters was very low in sucrose, suggesting their minor role in transport of this carbohydrate.

Our results demonstrate that *S. mutans* possesses inducible transporters for specific sugars and five PTS transporters that are consistently highly transcribed and presumably available for immediate uptake of the common dietary sugars. The capacity of *S. mutans* to rapidly transport and metabolize a wide range of sugars, whenever they become available, may be directly related to its survival in dental plaque and its cariogenic potential in humans.

ACKNOWLEDGMENTS

We thank Joseph J. Ferretti and Jeffrey Banas for discussions and critical reading of the manuscript. We also thank Norma McElwee and Gorana Savic for technical assistance.

This research was supported by National Institutes of Health grant P20RR018741.

REFERENCES

1. **Abranches, J., M. M. Candella, Z. T. Wen, H. V. Baker, and R. A. Burne.** 2006. Different roles of EIIABMan and EIIGlc in regulation of energy metabolism, biofilm development, and competence in *Streptococcus mutans*. J. Bacteriol. **188:**3748–3756.
2. **Abranches, J., Y. Y. Chen, and R. A. Burne.** 2003. Characterization of *Streptococcus mutans* strains deficient in EIIAB Man of the sugar phosphotransferase system. Appl. Environ. Microbiol. **69:**4760–4769.
3. **Abranches, J., Y. Y. Chen, and R. A. Burne.** 2004. Galactose metabolism by *Streptococcus mutans*. Appl. Environ. Microbiol. **70:**6047–6052.
4. **Ajdic, D., W. M. McShan, R. E. McLaughlin, G. Savic, J. Chang, M. B. Carson, C. Primeaux, R. Tian, S. Kenton, H. Jia, S. Lin, Y. Qian, S. Li, H. Zhu, F. Najar, H. Lai, J. White, B. A. Roe, and J. J. Ferretti.** 2002. Genome sequence of *Streptococcus mutans* UA159, a cariogenic dental pathogen. Proc. Natl. Acad. Sci. USA **99:**14434–14439.

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5. **Ajdic, D., I. C. Sutcliffe, R. R. Russell, and J. J. Ferretti.** 1996. Organization and nucleotide sequence of the *Streptococcus mutans* galactose operon. Gene **180:**137–144.
6. **Burne, R. A., and J. E. Penders.** 1992. Characterization of the *Streptococcus mutans* GS-5 *fruA* gene encoding exo-beta-D-fructosidase. Infect. Immun. **60:**4621–4632.
7. **Cote, C. K., D. Cvitkovitch, A. S. Bleiweis, and A. L. Honeyman.** 2000. A novel beta-glucoside-specific PTS locus from *Streptococcus mutans* that is not inhibited by glucose. Microbiology **146:**1555–1563.
8. **DaCosta, T., and R. J. Gibbons.** 1968. Hydrolysis of levan by human plaque streptococci. Arch. Oral Biol. **13:**609–617.
9. **Ellwood, D. C., P. J. Phipps, and I. R. Hamilton.** 1979. Effect of growth rate and glucose concentration on the activity of the phosphoenolpyruvate phosphotransferase system in *Streptococcus mutans* Ingbritt grown in continuous culture. Infect. Immun. **23:**224–231.
10. **Fukui, K., and T. Moriyama.** 1983. Effect of maltose on glucan synthesis by glucosyltransferases of *Streptococcus mutans*. Microbiol. Immunol. **27:**917–927.
11. **Gauthier, L., D. Mayrand, and C. Vadeboncoeur.** 1984. Isolation of a novel protein involved in the transport of fructose by an inducible phosphoenolpyruvate fructose phosphotransferase system in *Streptococcus mutans*. J. Bacteriol. **160:**755–763.
12. **Guggenheim, B., and J. J. Burkhardt.** 1974. Isolation and properties of a dextranase from *Streptococcus mutans* OMZ176. Helv. Odontol. Acta **18:**101–113.
13. **Hamada, S., and H. D. Slade.** 1980. Biology, immunology and cariogenicity of *Streptococcus mutans*. Microbiol. Rev. **44:**331–384.
14. **Hamilton, I. R., and H. Lebtag.** 1979. Lactose metabolism by *Streptococcus mutans*: evidence for induction of the tagatose 6-phosphate pathway. J. Bacteriol. **140:**1102–1104.
15. **Honeyman, A. L., and R. Curtiss III.** 1993. Isolation, characterization and nucleotide sequence of the *Streptococcus mutans* lactose-specific enzyme II (lacE) gene of the PTS and the phospho-beta-galactosidase (lacG) gene. J. Gen. Microbiol. **139:**2685–2694.
16. **Jagusztyn-Krynicka, E. K., J. B. Hansen, V. L. Crow, T. D. Thomas, A. L. Honeyman, and R. Curtiss III.** 1992. *Streptococcus mutans* serotype c tagatose 6-phosphate pathway gene cluster. J. Bacteriol. **174:**6152–6158.
17. **Kilic, A. O., A. L. Honeyman, and L. Tao.** 2007. Overlapping substrate specificity for sucrose and maltose of two binding protein-dependent sugar uptake systems in *Streptococcus mutans*. FEMS Microbiol. Lett. **266:**218–223.
18. **Liberman, E. S., and A. S. Bleiweis.** 1984. Transport of glucose and mannose by a common phosphoenolpyruvate-dependent phosphotransferase system in *Streptococcus mutans* GS5. Infect. Immun. **43:**1106–1109.
19. **Malke, H., K. Steiner, W. M. McShan, and J. J. Ferretti.** 2006. Linking the nutritional status of *Streptococcus pyogenes* to alteration of transcriptional gene expression: The action of CodY and RelA. Int. J. Med. Microbiol. **296:**259–275.
20. **Maryanski, J. H., and C. L. Wittenberger.** 1975. Mannitol transport in *Streptococcus mutans*. J. Bacteriol. **124:**1475–1481.
21. **Neron, S., and C. Vadeboncoeur.** 1987. Two functionally different glucose phosphotransferase transport systems in *Streptococcus mutans* and *Streptococcus sobrinus*. Oral Microbiol. Immunol. **2:**171–177.
22. **Old, L. A., S. Lowes, and R. R. Russell.** 2006. Genomic variation in *Streptococcus mutans*: deletions affecting the multiple pathways of beta-glucoside metabolism. Oral Microbiol. Immunol. **21:**21–27.
23. **Pfaffl, M. W.** 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Rese. **29:**2002–2007.
24. **Poolman, B., J. Knol, C. van der Does, P. J. Henderson, W. J. Liang, G. Leblanc, T. Pourcher, and I. Mus-Veteau.** 1996. Cation and sugar selectivity determinants in a novel family of transport proteins. Mol. Microbiol. **19:**911–922.
25. **Poy, F., and G. Jacobson.** 1990. Evidence that a low-affinity sucrose phosphotransferase activity in *Streptococcus mutans* GS-5 is a high-affinity trehalose uptake system. Infect. Immun. **58:**1479–1480.
26. **Reizer, J., and M. H. Saier, Jr.** 1994. A functional superfamily of sodium/solute symporters. Biochim. Biophys. Acta **1197:**133–166.
27. **Rosey, E. L., and G. C. Stewart.** 1992. Nucleotide and deduced amino acid sequences of the *lacR*, *lacABCD*, and *lacFE* genes encoding the repressor, tagatose 6-phosphate gene cluster, and sugar-specific phosphotransferase system components of the lactose operon of *Streptococcus mutans*. J. Bacteriol. **174:**6159–6170.
28. **Russell, R. R. B., J. Aduse-Opoku, I. C. Sutcliffe, L. Tao, and J. J. Ferretti.** 1992. A binding protein-dependent transport system in *Streptococcus mutans* responsible for multiple sugar metabolism. J. Biol. Chem. **267:**4631–4637.
29. **Saier, M. H., M. J. Fagan, C. Hoischen, and J. Reizer.** 1993. Transport mechanisms. ASM Press, Washington, DC.
30. **Sato, Y., F. Poy, G. R. Jacobson, and H. K. Kuramitsu.** 1989. Characterization and sequence analysis of the *scrA* gene encoding enzyme IIScr of the *Streptococcus mutans* phosphoenolpyruvate-dependent sucrose phosphotransferase system. J. Bacteriol. **171:**263–271.
31. **Schachtele, C. F., and J. A. Mayo.** 1973. Phosphoenolpyruvate-dependent glucose transport in oral streptococci. J. Dent. Res. **52:**1209–1215.
32. **Slee, A. M., and J. M. Tanzer.** 1982. Sucrose transport in *Streptococcus mutans*. Evidence for multiple tranport systems. Biochim. Biophys. Acta **692:**415–424.
33. **Tao, L., I. C. Sutcliffe, R. R. B. Russell, and J. J. Ferretti.** 1993. Cloning and expression of the multiple sugar metabolism (*msm*) operon of *Streptococcus mutans* in heterologous streptococcal host. Infect. Immun. **61:**1121–1125.
34. **Tao, L., I. C. Sutcliffe, R. R. B. Russell, and J. J. Ferretti.** 1993. Transport of sugars, including sucrose, by the Msm transport system of *Streptococcus mutans*. J. Dent. Res. **72:**1386–1390.
35. **Terleckyj, B., Willett, N. P., and G. D. Shockman.** 1975. Growth of several cariogenic strains of oral streptococci in a chemically defined medium. Infect. Immun. **11:**649–655.
36. **Vadeboncoeur, C.** 1984. Structure and properties of the phosphoenolpyruvate:glucose phosphotransferase system of oral streptococci. Can. J. Microbiol. **30:**495–502.
37. **Vadeboncoeur, C., and M. Pelletier.** 1997. The phosphoenolpyruvate:sugar phosphotransferase system of oral streptococci and its role in the control of sugar metabolism FEMS Microbiol. Rev. **19:**187–207.
38. **Walker, G. J., M. D. Hare, and J. G. Morrey-Jones.** 1983. Activity of fructanase in batch cultures of oral streptococci. Carbohydr. Res. **113:**101–112.
39. **Webb, A. J., and A. H. Hosie.** 2006. A member of the second carbohydrate uptake subfamily of ATP-binding cassette transporters is responsible for ribonucleoside uptake in *Streptococcus mutans*. J. Bacteriol. **188:**8005–8012.
40. **Wen, Z. T., C. Browngardt, and R. A. Burne.** 2001. Characterization of two operons that encode components of fructose-specific enzyme II of the sugar: phosphotransferase system of *Streptococcus mutans*. FEMS Microbiol. Lett. **205:**337–342.
41. **Yamashita, Y., Y. Tsukioka, K. Tomihisa, Y. Nakano, and T. Koga.** 1998. Genes involved in cell wall localization and side chain formation of rhamnose-glucose polysaccharide in *Streptococcus mutans*. J. Bacteriol. **180:**5803–5807.
42. **Zeng, L., Z. T. Wen, and R. A. Burne.** 2006. A novel signal transduction system and feedback loop regulate fructan hydrolase gene expression in *Streptococcus mutans*. Mol. Microbiol. **62:**187–200.

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