The global impact of the delta subunit RpoE of the RNA polymerase on the proteome of *Streptococcus mutans*

Running title: The role of RpoE on *S. mutans* proteome

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List of abbreviations:

ROS: reactive oxygen species; 2-DE: two-dimensional gel electrophoresis; TCS: two component signal transduction system; BCAAs: branched chain amino acids; MLF: malolactic fermentation; MSM: multiple sugar transport and metabolism.

Summary

Transcriptional specificity in low G+C Gram positive bacteria is maintained by RpoE, the delta subunit of the RNA polymerase. Here, we studied the role of RpoE on the proteome level in the human dental pathogen *Streptococcus mutans* by comparing the Δ*rpoE* mutant to the wild type under five conditions: (0) logarithmic growth, (1) early stationary phase, (2) acid stress, (3) oxidative stress, and (4) combined acid and oxidative stress.

A total of 280 cellular protein spots were reproducibly detected, of which 97 differentially expressed protein spots were identified by MALDI-TOF MS. Lack of RpoE caused down-regulation of proteins for carbohydrate metabolism and energy production, including the phosphoglucomutase PGM, the phosphopentomutase DeoB, and the pyruvate formate-lyase Pfl. The Δ*rpoE* mutant had extensive changes in the abundance of proteins involved in acid and oxidative tolerance, protein turn over, and chaperones, at log phase in the absence of stress, suggesting a potential internal stress. In addition, the mutant had a reduced amount of proteins for adaptation responses, e.g. the multiple sugar transport and metabolism enzymes required for entering early stationary phase, and the proteins for stress defense mechanisms and glycolysis under oxidative stress. Comparison of the proteome data with the corresponding transcriptome data suggested that the effects were the result of altered transcriptional and post-transcriptional regulation. The data are consistent with the reduced transcriptional specificity of the RNA polymerase in the Δ*rpoE* mutant and suggest a general impact, but not a specific regulatory role of RpoE for stress adaptation.

Introduction

Transcription is the first step in gene regulation, and bacterial cells normally have one housekeeping sigma (σ) factor and several alternative σ factors to activate different sets of genes in response to environmental changes (Gruber & Gross, 2003). The delta (δ) subunit of the RNA polymerase, RpoE, is a unique protein in low G+C Gram positive bacteria (Firmicutes) (Jones *et al.*, 2003). RpoE is suggested to maintain transcriptional specificity by reducing unspecific binding of RNA polymerase to DNA and by accelerating the core enzyme recycling (Achberger *et al.*, 1982;Achberger & Whiteley, 1981;Juang & Helmann, 1994). The amino terminal part of RpoE is conserved and binds to the RNA polymerase (Lopez de Saro *et al.*, 1995), while the carboxyl terminus is highly unstructured and acidic, and is predicted to displace nucleic acid from the RNA polymerase (Lopez de Saro *et al.*, 1995). Sequence alignment suggests that the σ^A factor and RpoE subunit in Gram-positive bacteria (e.g. *Bacillus subtilis*) together may contain the activity of the σ^{70} factor from Gram-negative bacteria (e.g. *Escherichia coli*) (Lopez de Saro *et al.*, 1995). The physiological role of RpoE has been studied only in a few reports. In *B. subtilis*, which contains 17 alternative σ factors (Gruber & Gross, 2003), the Δ*rpoE* mutant revealed only an extended lag phase of growth and altered cell morphology (Lopez de Saro *et al.*, 1999). However, in *Staphylococcus aureus*, which contains two alternative σ factors: σ^B and σ^H (Tao *et al.*, 2010), disruption of the *rpoE* homologue resulted in a survival defect under amino acid starvation and acid stress (Watson *et al.*, 1998). Moreover, RpoE was demonstrated to affect the virulence in *Streptococcus agalactiae* (Jones *et al.*, 2003;Seepersaud *et al.*, 2006), which contains only one secondary σ factor ComX functioning as a competence-specific regulator (Luo & Morrison, 2003;Opdyke *et al.*, 2001). Since RpoE contains partial functions of a σ factor, it could be more important in *Staphylococcus* and *Streptococcus*, which contain less alternative σ

68 factors. Especially, *Streptococcus sp.* contain no global stress regulator such as σ^B of other
69 Gram-positive bacteria to induce specific stress responses (Hecker *et al.*, 2007). Thus, it is very
70 interesting to study how *Streptococcus sp.* cope with stress without a global stress regulator.

71 *Streptococcus mutans*, a facultative anaerobic bacterium, is considered as an important pathogen
72 responsible for human dental caries (Ajdic *et al.*, 2002;Russell, 2008). It metabolises various
73 carbohydrates to produce acidic products, and forms biofilms (plaque) on the tooth surface,
74 which are the main reasons for tooth decay (Ajdic & Pham, 2007;Hojo *et al.*, 2009). *S. mutans*
75 has developed complex regulatory mechanisms to survive in the oral environment, which is
76 characterized by quick fluctuations of sources and concentrations of carbohydrates, as well as by
77 changing pH and redox state (Lemos *et al.*, 2005;Lemos & Burne, 2008).

78 To cope with fluctuating pH, *S. mutans* is well equipped with acid defense systems, which have
79 been studied extensively: the F_1F_0-ATPase pumps protons out of the cell (Lemos *et al.*, 2005);
80 the malolactic fermentation (MLF) transforms malate to the weaker acid lactic acid and CO_2 to
81 raise the intracellular pH (Lemme *et al.*, 2010;Sheng & Marquis, 2007); and the agmatine
82 deiminase system produces ammonia and ATP to alkalinize the cytoplasmic pH (Griswold *et al.*,
83 2006). Moreover, various TCSs, especially VicRK, LiaSR, and CiaHR, have been shown to be
84 required for acid adaptation (Biswas *et al.*, 2008;Gong *et al.*, 2009).

85 Reactive oxygen species (ROS) can be generated by oral hygiene products or by microbial
86 metabolism in the presence of oxygen (O_2) in the oral cavity, including superoxide anion (O_2^-),
87 hydrogen peroxide (H_2O_2), and hydroxyl radical ($HO^{\cdot}$) (Marquis, 2004). Compared to acid stress,
88 oxidative stress caused by H_2O_2 can be harsh for *S. mutans*, as it does not contain catalase, a key
89 enzyme required for oxidative stress protection (Higuchi *et al.*, 2000). However, a large number

of other ROS defense mechanisms have been found: the superoxide dismutase (SodA) detoxifies O_2^- by converting it to O_2 and H_2O_2 (Nakayama, 1992;Poyart *et al.*, 2001;Thomas & Pera, 1983); the H_2O_2-forming NADH oxidase Nox-1 functions together with the peroxidase AhpC, while the H_2O-forming NADH oxidase Nox-2 catalyzes directly the reduction of O_2 to H_2O (Higuchi *et al.*, 2000;Poole *et al.*, 2000); the iron-binding enzyme Dpr regulates the intracellular iron level to reduce the generation of ROS through the Fenton Reaction (Higuchi *et al.*, 2000); glutathione reductase GshR and thioredoxin reductase TrxB repair oxidative damage in proteins (Carmel-Harel & Storz, 2000;De Angelis & Gobbetti, 2004;Jansch *et al.*, 2007). Besides the known antioxidant enzymes, the two component signal transduction systems (TCSs) VicRK and ScnRK, and the response regulator RR11 (SMU.1547c) of the TCS HK11/RR11 were shown to be required for protection of *S. mutans* from oxidative stress (Chen *et al.*, 2008;Deng *et al.*, 2007a;Perry *et al.*, 2008). Moreover, the serine protease ClpP (Deng *et al.*, 2007b), the trigger factor RopA (Wen *et al.*, 2005), a putative oxidoreductase (SMU.2115) (Abranches *et al.*, 2006), a putative surface-associated protein BrpA (Wen *et al.*, 2006), and a putative phosphatase (SMU.1297) (Zhang & Biswas, 2009) were also shown to be involved in the oxidative stress tolerance.

Two-dimensional gel electrophoresis (2-DE) based proteome technology is a powerful tool for understanding the global response of oral bacteria to environmental challenges at the protein level (Len *et al.*, 2003;Macarthur & Jacques, 2003;Renzone *et al.*, 2005). The effect of pH on the proteome of *S. mutans* has been well studied. Changes in metabolic pathways, including glycolysis, alternative acid production and synthesis of branched chained amino acids (BCAAs) were identified in *S. mutans* during growth at low pH (Len *et al.*, 2004a;Renzone *et al.*, 2005) on the proteome level. Moreover, proteins involved in DNA replication, transcription, translation,

protein folding and cleavage were up-regulated under acidic conditions (Len *et al.*, 2004b;Wilkins *et al.*, 2002). Oxidative stress has been studied much less. Svensäter et al. studied the adaptation of *S. mutans* to diverse stresses, including oxidative, acid, starvation, salt and heat stresses, on the proteome level (Svensater *et al.*, 2000). Treatment with 2 mM H_2O_2 for 30 min resulted in 69 protein spots with increased abundance and 24 protein spots with decreased abundance. Unfortunately, these protein spots were not identified.

To evaluate the role of RpoE in *S. mutans*, a Δ*rpoE* mutant was constructed in our previous work (Xue *et al.*, 2010). The mutant showed impaired growth, altered biofilm architecture, and reduced resistance against acid and oxidative stresses. Genetic complementation of the mutant with the *rpoE* gene in *trans* showed reversal to the wild type phenotype (e.g. growth, biofilm formation, and stress tolerance), indicating that secondary mutations were not present. Loss of RpoE cause massive changes in the expression of genes and intergenic regions (Xue *et al.*, 2010). In addition, the mutant had increased virulence related traits, including increased adherence to human extracellular matrix components, a broader spectrum of carbon substrates that were respired, and it was resistant to a large number of toxic compounds (Xue *et al.*, 2011). Here we investigated the role of RpoE on the proteome of *S. mutans* during log and early stationary phase of growth, acid stress (pH 5), oxidative stress (H_2O_2) and combined acid/oxidative stress. A comparison of proteome and transcriptome changes of both wild type and mutant under all tested conditions was also performed.

Materials and Methods

Chemicals and enzymes.

Amberlite, amidosulfobetaine-14 (ASB-14), 3[(3-Cholamidopropyl)dimethylammonio]-propanesulfonic acid (CHAPS), chloramphenicol, Coomassie Blue G-Colloidal Concentrate, diethanolamine, dithiothreitol (DTT), ethylenediaminetetraacetic acid (EDTA), phenylmethylsulfonyl fluoride (PMSF), thiourea, Triton X-100, trizma base, trypsin, and urea were obtained from Sigma-Aldrich. Roti-Aqua-Phenol was obtained from Roth. Ammonium persulfate was obtained from MERCK. Immobiline DryStrips (pH 4-7, 24 cm), IPG buffer (pH 4-7, pH 3-10) were purchased from GE Healthcare. Sypro-RuBPS (Ruthenium (II) tris bathophenantroline disulfonate) was self prepared according to the method described by Rabilloud et al (Rabilloud *et al.*, 2001).

Bacterial strains, plasmids and growth conditions.

Streptococcus mutans UA159 wild type and the Δ*rpoE* mutant (deletion of *rpoE* gene by replacing the coding sequence with erythromycin cassette) (Xue *et al.*, 2010) strains were grown in Todd Hewitt broth (Becton Dickinson) supplemented with 1% yeast extract (THBY medium) at 37°C aerobically (5% CO_2 enriched). Erythromycin was included where indicated at a final concentration of 10μg/ml for the Δ*rpoE* strain.

Preparation of whole-cell protein lysates.

Overnight cultures of the *S. mutans* wild type and the Δ*rpoE* mutant were 1:20 diluted in fresh THBY medium buffered with 75 mM phosphate buffer at pH 7.5 and grown at 37 °C aerobically until OD_{600} reached about 0.5. An aliquot of each culture at log phase was withdrawn and used as a control (condition 0) for comparative proteome analysis. The remaining log phase cells were

collected by centrifugation (12,000 rpm, 30 sec) and incubated in buffered THBY medium for 2 hours as follows: (1) at pH 7.5; (2) at pH 5.0; (3) at pH 7.5 + 2 mM H_2O_2; (4) at pH 5.0 + 2 mM H_2O_2. Chloramphenicol at a concentration of 50 μg/mL and 1 mM PMSF were added immediately to the cultures before centrifugation at 5,000 rpm and 4°C for 10 min. The pellets were washed twice in cold PBS (pH 7.4) containing 1 mM EDTA, 50 μg/ml chloramphenicol and 1 mM PMSF. After centrifugation, pellets were stored at -70°C till use. For each sample, two biological replicates were analyzed.

Whole-cell protein lysate preparation was carried out according to methods described before (Wang *et al.*, 2005;Wang *et al.*, 2006) with some modifications. Briefly, cell pellets were resuspended in 1.5 ml lysis buffer (OD_{600} about 30) containing 7 M urea, 2 M thiourea, 2% (w/v) CHAPS, 40 mM DTT, 0.5% (w/v) ASB-14, 0.5% (w/v) Triton X-100, and 1mM Pefabloc. Cells were disrupted using a FastPrep-24 high-speed homogenizer (MP Biomedicals) with the Lysis Matrix C tubes containing 0.1 mm silica spheres. Disruption was performed for 8 cycles of 1 min at a speed of 6.0 m/sec with 5 min intervals between each cycle. Following centrifugation at 13,000 rpm for 10 min, the supernatants of the samples were collected and purified by phenol precipitation (Carpentier *et al.*, 2005;Saravanan & Rose, 2004). After resuspension of the pellets in the lysis buffer, protein concentrations were determined by the 2-D Quant Kit according to the manufacturer's instruction (GE Healthcare).

Two-dimensional gel electrophoresis (2-DE).

For each sample, 250 μg of each whole-cell protein lysate in the rehydration buffer containing 7 M urea, 2 M thiourea, 2% (w/v) CHAPS, 40 mM DTT, 0.5% (w/v) amidosulfobetaine-14 (ASB-14), 0.5% (w/v) Triton X-100, and 0.5% v/v IPG buffer pH 4-7, was loaded onto an Immobiline DryStrip gel (24 cm, pH 4-7). The first dimension isoelectric focusing was run on an IPGPhor

177 Isoelectric Focusing System (GE Healthcare) at 20°C for a total of 100.5 kVh (30 V for 6 h,
178 followed by 60 V for 6 h, 200 V for 1 h, 500 V for 1 h, 1000 V for 1 h, gradient to 8000 V within
179 30 min and 8000 V for 12 h). After focusing, the proteins were reduced by incubating the IPG
180 strips with 1% w/v DTT for 15 min and then alkylated with 2.5% w/v iodoacetamide for 15 min in 15
181 ml equilibration buffer (50 mM Tris-HCl, pH 8.8, 6 M urea, 30% w/v glycerol, 2% w/v SDS). The
182 strips were then transferred to 12.5% SDS-PAGE gels for the second dimension using a vertical
183 slab separation unit (Ettan Dalt II System, GE Healthcare) with running conditions 2 W/gel for 1
184 h and then 15 W/gel until the bromophenol blue dye reached the bottom of the gel. Gels were
185 fixed overnight in a fixing solution (10% v/v glacial acetic acid, 30% v/v ethanol, 60% v/v
186 MilliQ water), washed 3 times (30 min each time) in washing solution (20% v/v ethanol, 80%
187 v/v MilliQ water). Gels were transferred to Sypro-RuBPS staining solution (2 μM Sypro-RuBPS
188 fluorescent dye in washing solution, 250 ml/gel) and stained in the dark overnight. After
189 equilibration in MilliQ water 2 times (10 min each time), the gels were destained (40% v/v
190 ethanol, 10% v/v acetic acid) for 15 hours.

191 **Image acquisition and analysis.**

192 Destained gels were scanned with a CCD based Fujifilm LAS-1000 image analyzer using the
193 parameters described before (Wang *et al.*, 2006). The images from 24 gels (2 biological
194 replicates and 0-2 technical replicates for both wild type and the mutant strain in each of five
195 conditions) were analyzed by using the Progenesis SameSpots (Non-linear dynamic, UK)
196 software (version 3.3). Image alignment was based on automatic default analysis and manual
197 editing of matched vectors. Spot detection was performed simultaneously across all images,
198 obtaining a master list of detected spots and corresponding spot boundaries for quantification of
199 protein spot volume. For calibration of gel-gel variation and for spot volume normalization, an

improved method, instead of the traditional Total Spot Volume approach, was used (http://www.nonlinear.com/support/progenesis/samespots/faq/normalisation.aspx). Normalized spot volumes were used for comparison. P values of differentially expressed proteins were calculated according to the one way Anova test. The whole dataset of each group was used for principle component analysis to have a general overview of data variation, by using the Bioconductor package written in R language (http://www.r-project.org/).

Protein identification.

2-DE gels were further stained using Brilliant Blue G-Colloidal Concentrate according to the manufacturer's instruction. Protein spots of interest (fold of changes >= 2 and $P<0.05$ in at least one comparison) were excised from the gels and subjected to tryptic digestion according to a method described previously (Wang *et al.*, 2003;Wang *et al.*, 2005) with some modifications. Briefly, protein spots were excised to 96-well microtiter plates and rinsed with acetonitrile, washed twice with MilliQ water and 50 mM NH_4HCO_3 in turn, and destained by washing in 50 mM NH_4HCO_3 and acetonitrile alternatively for several times until the Coomassie dye became nearly invisible. Then gel pieces were dehydrated with acetonitrile and dried completely by lyophilisation. After in-gel tryptic digestion and extraction by 5% formic acid and actonitrile sequentially, the pooled peptide extracts were concentrated by lyophilisation and spotted on Prespotted Anchorchip (PAC 384/96 CHCA, Bruker, Germany) according to the manufacturer's instruction.

Subsequently, the tryptic peptides were analyzed by MALDI-TOF MS with a Bruker Ultraflex time-of-flight mass spectrometer (Bruker Daltonics GmbH). Peptide masses obtained from MALDI-TOF MS analysis were used for protein identification by peptide mass fingerprinting (PMF) using the MASCOT program licensed in-house to search the strain-specific protein

223 database “smu” which was downloaded from NCBI (URL:
224 ftp://ftp.ncbi.nih.gov/genomes/Bacteria/Streptococcus_mutans/NC_004350.faa) and installed on
225 our local Mascot server. Parameters for protein identification included a mass tolerance of 100
226 p.p.m. and allowed up to one missed cleavage per peptide while taking into consideration
227 carboxymethyl (fixed modification) and methionine oxidation modifications (variable
228 modification). Protein score is -10*Log(P), where P is the probability that the observed match is
229 a random event. Protein scores greater than 45 are considered as positive results ($p<0.05$).

Results and Discussion

Experimental set-up.

The proteome of *S. mutans* wild type and the Δ*rpoE* mutant was studied under five conditions: (0) Log phase of growth immediately before treatment; 2 hour treatment at (1) pH 7.5 (culture reached early stationary phase); (2) pH 5 (acid stress); (3) 2 mM H_2O_2 (oxidative stress); and (4) pH 5 and 2 mM H_2O_2 (a combination of both stresses). It was the aim of our work to study the adaptation to the respective stresses. Samples were analysed after 2 h of stress treatment, since our previous results had shown that 2 hours of incubation at relatively mild stress conditions (pH 5 and 2 mM H_2O_2) provided protection for both strains for the subsequent killing stresses at pH 3 and 20 mM H_2O_2, respectively (Xue *et al.*, 2010). Growth and viability of the wild type and the Δ*rpoE* mutant under these conditions are shown in supplementary figure S1. Acid stress resulted in slower growth of both strains. Oxidative stress caused a reduction in final optical density (OD) in the wild type at pH 7.5; however, in combination with a pH of 5 it arrested its growth completely. The Δ*rpoE* mutant was unable to grow under oxidative stress, both at pH 7.5 and at pH 5.0. Cells remained viable, however, as shown by the colony forming units (CFU) data. The growth arrest under combined oxidative/acid stress for both wild type and Δ*rpoE* mutant was clearly reflected in a strong decrease of the CFU values (figure S1). Under peroxide stress alone, the OD of the wild type suggested weak growth, but the CFU values were similar to those obtained under combined oxidative/acid stress. The OD values could have been affected by cell lysis or different surface properties of the cells that had been subject to oxidative stress; on the other hand, the disruption of cell chains by vortexing before plating might have been different in stressed cells and thus have biased the CFU determination. Adaptation may occur in the absence

of growth, for example, the growth-arrested *E. coli* cells produce the universal stress protein UspA, which is suggested to protect cells from diverse stresses, such as H_2O_2, osmotic stress, and starvation (Kvint *et al.*, 2003;Nystrom & Neidhardt, 1994). The whole-cell protein extracts were subjected to two-dimensional gel electrophoresis (2-DE). For each strain in each condition, 2-4 replicates comprised of 2 biological and 0-2 technical replicates were investigated, resulting in a total of 24 gels. 280 protein spots were detected in the pH range of 4-7 and molecular weight between10 -100 kD. After spot detection, calibration of gel-gel variation and spot volume normalization, protein abundance, fold of change values and significance were calculated. The technical replicates for the wild type and the mutant under the control condition (log phase) revealed a correlation coefficient above 0.99. The correlation coefficient between biological replicates was above 0.99 for all datasets, except for M 2 (the Δ*rpoE* mutant at pH 5), thus the reproducibility was high. Because the M 2 biological replicates displayed a lower correlation coefficient (0.97), more replicates (two technical replicates of each biological replicate) were performed for the mutant under this condition.

Principal component analysis of the complete dataset.

A principal component analysis was performed on the protein abundance data of the total 280 protein spots for all analyzed samples to determine similarities and differences between the samples. As shown in Fig. 1, biological replicates grouped very closely together, as expected from the correlation analysis described above. The datasets of the wild type and the Δ*rpoE* mutant were separated by the first three components, which contributed to about 50% of the total variance. The protein abundance patterns of the wild type at log and early stationary growth phase and under acid stress were similar, since *S. mutans* has a high acid tolerance (Lemos *et al.*, 2005;Lemos & Burne, 2008). The H_2O_2 stress had a remarkable impact on the wild type,

indicated by the large distance of the H_2O_2 dataset (at ph 7.5) from the others. The combined acid and oxidative (H_2O_2) stress had a smaller effect on the proteome, most likely due to the growth arrest which occurred under this condition. In the Δ*rpoE* mutant, the distance between the datasets of any of these adaptive conditions and log phase was reduced, indicating that loss of RpoE resulted in an inability of the mutant to respond strongly and specifically to the environmental changes. One hundred and four protein spots whose abundance had a fold of change >=2 ($p < 0.05$) in at least one comparison (mutant compared to wild type, or stress conditions compared to log phase) were excised and digested by trypsin, and 97 protein spots were successfully identified by MALDI-TOF (see supplementary Table S1 for a complete list of identified proteins). For the analysis described below, the abundance changes of all identified protein spots with fold of change >=1.3, $p<0.05$ were considered as significant changes.

Effect of RpoE on *S. mutans* proteome under various conditions.

The effect of the *rpoE* mutation on the proteome of *S. mutans* was analyzed by directly comparing the mutant to the wild type under the five conditions described above. The data are listed in Tables 1-3 and Tables S2-3.

<u>General effect of RpoE on proteins involved in carbohydrate metabolism and energy production.</u>

In comparison to the wild type, the Δ*rpoE* mutant showed a reduction in the abundance of proteins for carbohydrate metabolism and energy production under all investigated conditions. This is consistent with the slower growth of the mutant in rich medium as shown previously (Xue *et al.*, 2010) and in this study (supplementary figure S1), since efficient sugar metabolism and the resulting energy production is important for the fast growth of *S. mutans*.

296 Especially, the enzyme phosphoglucomutase PGM was down-regulated in the mutant under all
297 conditions. PGM catalyzes the interconversion of glucose-6-phosphate and glucose-1-phosphate,
298 and is a key enzyme in the metabolic flux between glycolysis and exopolysaccharide production
299 (Levander & Radstrom, 2001). PGM deficiency results in pleiotropic effects in Streptococci, e.g.
300 altered cell wall muropeptide pattern and a lower teichoic acid content (Bizzini *et al.*, 2007),
301 reduced capsule expression (Hardy *et al.*, 2000), growth defect (Bizzini *et al.*, 2007;Hardy *et al.*,
302 2000) and reduced virulence (Bizzini *et al.*, 2007;Buchanan *et al.*, 2005). Thus, the reduction of
303 PGM in the mutant may affect the cell wall composition and exopolysaccharide production,
304 which partially explains its altered surface properties and biofilm structure observed in our
305 previous results (Xue *et al.*, 2011).

306 The phosphopentomutase DeoB had a reduced abundance under all conditions except the
307 peroxide stress (which was due to the reduction of DeoB in the wild type under this condition).
308 This is consistent with the down-regulation of genes for histidine biosynthesis in the Δ*rpoE*
309 mutant (Xue *et al.*, 2010). DeoB is involved in the pentose phosphate pathway, and it catalyzes
310 the intramolecular transfer of the phosphate group between Ribose-1P and Ribose-5P. The latter
311 is a direct precursor of phosphoribosyl pyrophosphate, which is an important intermediate for
312 histidine and purine/pyrimidine biosynthesis (Tozzi *et al.*, 2006).

313 Pyruvate is an important intermediate in glycolysis, and can be converted to diverse acid end
314 products in *S. mutans* (Korithoski *et al.*, 2008). The fermentation of pyruvate regenerates NAD^+,
315 which is important for glycolysis. Proteins for pyruvate metabolism and energy production were
316 down-regulated in the mutant. For example, the pyruvate formate-lyase Pfl which converts
317 pyruvate to formate had reduced abundance in the mutant under all conditions except the
318 peroxide stress. Pfl is extremely oxygen-sensitive, and its activity is irreversibly lost by exposure

to air (Yamada *et al.*, 1985). Accordingly we found less of the Pfl enzyme in the wild type under peroxide stress; by contrast, the amount of lactate dehydrogenase Ldh for another branch of pyruvate metabolism was increased.

Effect of RpoE in log phase of growth.

The abundance of proteins for acid stress, oxidative stress, protein turnover and chaperones was changed in the mutant during log phase of growth (Table 1), suggesting that loss of RpoE may cause internal stress. For example, the abundance of malate dehydrogenase MleS from the malolactic fermentation (MLF) acid defense system was reduced, while the amount of the branched chain amino acids (BCAAs) biosynthesis enzymes (IlvC, IlvD, and IlvE) together with the glutamine synthetase GlnA, was increased in the Δ*rpoE* mutant at log phase. BCAAs biosynthesis enzymes are also part of the acid defense system in *S. mutans* (Len *et al.*, 2004a).

Effect of RpoE in early stationary phase.

In addition to the reduction in the abundance of proteins for carbohydrate metabolism and energy production, which was found under all conditions, 4 proteins (MsmK, GtfA, DexB, and GalE) for the multiple sugar transport and metabolism (MSM) system were down-regulated in the mutant compared to wild type cells when entering early stationary phase (Table 2). The MSM system is an ATP-binding cassette (ABC) transporter involved in uptake and metabolism of a wide range of sugars, including trisaccharides (raffinose), disaccharides (sucrose, melibiose), and monosaccharides (glucose, fructose, galactose) (Abranches *et al.*, 2004;Tao *et al.*, 1993). Indeed, the relatively reduced amount of MSM enzymes in the mutant was not caused by down-regulation, but was the result of a strong up-regulation of these enzymes in the wild type (Table S4). The induction of MSM enzymes for sugar metabolism at stationary phase allows cells to

341 metabolize alternative, less favorable sugars that are taken up from the environment after the
342 primary substrates have been depleted. Thus, the lack of such an induction in the mutant could
343 have contributed to its reduced final optical density. However, the utilization of carbon sources
344 could be affected strongly by medium and cultivation conditions. In contrast to the growth
345 condition used in this study (a rich medium and aerobic cultivation with additional CO_2), when
346 we used a minimal medium and aerobic cultivation condition, the mutant showed increased
347 metabolic activity on 20 additional carbon sources in comparison to the wild type (Xue *et al.*,
348 2011).

349 Effect of RpoE under acid stress.

350 No significant difference was found in the amount of proteins of the acid defense mechanisms
351 when directly comparing the mutant to the wild type at pH 5 (Table S2). Nevertheless, the
352 weaker acid tolerance of the mutant (Xue *et al.*, 2010) could be due to the overall reduced fitness
353 of the mutant. For example, 7 proteins for translation showed altered expression in the mutant in
354 comparison to the wild type under acid stress, 5 of which were down-regulated.

355 Effect of RpoE under oxidative stress.

356 The oxidative stress defense proteins, e.g. the superoxide dismutase SodA, the NADH oxidase
357 Nox-1, and the glutathione reductase GshR, as well as the proteins for glycolysis (Eno, PykF,
358 PfkA, GapC, FbaA) were down-regulated in the mutant in comparison to the wild type in the
359 adaption to the peroxide stress (Table 3). Again, the reduced abundance of these enzymes in the
360 mutant was due to their strong induction in the wild type, rather than a down-regulation in the
361 mutant (Table S6). Up-regulation of glycolytic enzymes upon exposure to peroxide has also been
362 reported in *Salmonella enterica* (Kim *et al.*, 2010). The induction of glycolysis could provide

energy (ATP) and NADH, which are necessary for the elimination of H_2O_2 by NADH oxidases (Higuchi *et al.*, 2000). The lack of induction of the above proteins for oxidative stress defense could contribute to its deficiency in peroxide stress tolerance (Xue *et al.*, 2010) and the observed growth arrest (figure S1).

Effect of RpoE under combined acid and oxidative stress.

The differences between the mutant and the wild type under combined acid and oxidative stress were smaller than those observed under peroxide stress alone. For example, only one oxidative defense protein, SodA, was down-regulated in the mutant (Table S3), and the reduction in the fold of change was less than under peroxide stress. Instead of 5 glycolytic proteins which were down-regulated in the mutant under peroxide stress, only 3 isoforms of the glyceraldehyde-3-phosphate dehydrogenase GapC showed reduced expression in the mutant under combined stress conditions. The reduced differences between the two strains were likely due to the growth arrest of both strains under this condition.

Effect of RpoE on stress adaptation in *S. mutans.*

Because the *rpoE* mutation has fundamental effects on *S. mutans* during log phase of growth (the starting point for the other 4 conditions), e.g. changes in proteins related to carbohydrate metabolism, energy production, and stress responses, we normalized the other 4 conditions to the logarithmic growth, and compared the normalized values of the mutant to those of the wild type. This allowed us to detect changes which were specific to the applied stress condition for the RpoE mutant. The data are listed in Tables S4-S7. Interestingly, the mutant displayed very similar changes as the wild type, although the extent of change was lower in the mutant. For example, when comparing the early stationary phase proteome to the log phase proteome, the

385 amount of 5 proteins (MsmK, GtfA, DexB, GalK and GalE) from the MSM system was strongly
386 increased in the wild type, 3 of these proteins (MsmK, DexB, and GalK) were similarly
387 increased in the mutant, although to a much weaker extent (Table S4). Another example are the
388 oxidative defense proteins (SodA, GshR, Nox-1) and glycolytic enzymes (PfkA, GapC, GapN,
389 Eno, PykF). Their protein amount was increased in both strains under oxidative stress, but the
390 increase was weaker in the mutant (Table S6). These data suggest that the mutant specifically
391 responded to environmental changes, however, probably due to the loosened transcriptional
392 specificity, it could not produce enough protein to fully execute the corresponding functions.
393 Thus, RpoE does not activate a specific stress adaptation mechanism in *S. mutans*. By contrast,
394 the loosened transcriptional specificity due to lack of RpoE is sufficient to explain the proteome
395 changes under stress.

396 **Comparison of proteome and transcriptome analysis.**

397 The experimental setup for the proteome study presented here was identical with that of the
398 transcriptome study reported previously (Xue *et al.*, 2010), allowing us to compare the data from
399 both levels of regulation. In our previous study, the transcriptome data of the Δ*rpoE* mutant were
400 directly compared to the wild type under the same condition. A total of 550 differentially
401 expressed genes (fold of changes >= 2; $P < 0.005$) were found when all experimental conditions
402 were combined. Therefore, we previously focused on a core set of 24 genes which were
403 influenced by lack of RpoE under all investigated conditions. The complete operon for MLF, the
404 histidine biosynthesis operon, and genes influencing biofilm formation and antibiotic resistance
405 were among the core genes.

406 In the proteome study presented here, only cytoplasmic proteins with sufficient abundance,
407 molecular weight between 10-100 kDa and pI 4-7 were detected on the gels. Ninety-seven
408 significantly changed protein spots (fold of changes >= 2; $P < 0.05$) were identified and used for
409 further analysis, which is a much smaller dataset in comparison to the transcriptome. In the
410 proteome study, 2 proteins (MleS and OxdC which are part of MLF) from the core set of 24
411 genes were down-regulated as expected. The corresponding genes showed the highest fold
412 change values of all the core genes (Xue *et al.*, 2010). The remaining proteins had a higher or
413 lower pI or molecular weight (e.g. histidine synthesis enzymes, antibiotic resistance proteins), or
414 they were membrane bound or extracellular proteins (e.g. glucosyltransferases GtfB and GtfC,
415 glucan binding protein GpgC) and thus were not detectable in our proteome study. However,
416 proteome data provides information from another level of regulation, which is important for the
417 interpretation of the data. For example, the extensive changes in the expression of genes and
418 production of proteins functioning in the carbohydrate metabolism and energy production were
419 observed in both the transcriptome and proteome study. Moreover, the reduced abundance of
420 Pgm and DeoB enzymes found in the proteome study provides new evidence for the altered
421 biofilm structure observed previously and the reduced histidine metabolism related gene
422 expression of the mutant found in the transcriptome analysis, respectively.

423 To provide a systematic comparison of both levels of analysis, the corresponding gene
424 expression values of each protein are shown in Tables 1-3, and supplementary Tables S2 – S7.
425 The percentage of proteins which showed corresponding, contradictory, or no change in gene
426 expression was calculated by direct comparison of the mutant to the wild type (Table 4).

427 About 50 % of the differentially expressed proteins had correlated changes on the transcriptome
428 level (except for the peroxide stress), suggesting that the effect of RpoE on transcriptional

specificity was extended to the proteome. A low correlation between proteome and transcriptome changes was seen under the peroxide stress. Several enzymes for carbohydrate metabolism and energy production revealed only minor or no change in gene expression. Proteins for oxidative stress defense and general stress were up-regulated while the corresponding genes were down-regulated. The half-lives of proteins are much longer than the half-lives of mRNA (Evguenieva-Hackenberg & Klug, 2011); the oxidative stress related mRNAs may have been produced at high concentrations immediately after application of the peroxide stress, but had already been degraded 2 hours later when the corresponding proteins where still present in the cells. Thus, the inconsistent changes between mRNA and protein amount may have been caused by sampling at the two hour time point only. Strikingly low correlations between the levels of mRNA and proteins have, however, been frequently observed in integrated proteomic and transcriptomic studies (Evguenieva-Hackenberg & Klug, 2011). Posttranscriptional and posttranslational regulatory mechanisms like changes in mRNA stability, protein stability or protein degradation may account for this observation. The complexity of control of the amount of a single metabolic enzyme, β-galactosidase, is shown by the archetypical lac operon, the regulation of which, although it is perhaps the best studied operon in bacteria, is still not fully understood (Santillan & Mackey, 2008). A further layer of complexity is added by novel studies of gene expression and protein abundance on the single cell level. A genomwide study of protein and mRNA copy number in *E. coli* on the single cell level found that many transcripts were present in one copy per cell only and that protein and mRNA copy number were uncorrelated for any given gene (Taniguchi *et al.*, 2010). It should also be considered that a clonal population of *S. mutans* can segregate into phenotypically distinct subpopulations with entirely different transcriptomes and resultant proteomes (Lemme *et al.*, 2011).

Interestingly, some of the proteome changes under oxidative stress were most probably caused by direct action of the hydrogen peroxide on the enzymes. We observed that several proteins (e.g. SodA, GshR, GapC) displayed multiple isoforms with more acidic isoelectric points (pI). The level of these types of isoforms was elevated under peroxide stress, while that of the more neutral isoforms was less increased or even decreased. Acidic pI shifts of peroxiredoxin proteins have been reported in mammalian cells after peroxide treatment (Rabilloud *et al.*, 2002) and are due to an additional negative charge caused by oxidation of the active site cysteine to cysteic acid. A similar oxidation effect has also been reported in *Staphylococcus aureus*, where proteins that contained cysteine in the active site, e.g. glyceraldehyde-3-phosphate dehydrogenase (GapDH) or alkylhydroperoxide reductase (AhpC) displayed acidic gel shifts (Rabilloud *et al.*, 2002;Weber *et al.*, 2004;Wolf *et al.*, 2008). Thus, for proteins that contain cysteine in the active site, for example, the glutathione reductase GshR, and the glyceraldehyde-3-phosphate dehydrogenase GapC, the reason for the acidic pI shift found in this study could be the oxidation of cysteine residues. Shifts in acidic pI can also result from other post-translational modifications, such as phosphorylation (Rabilloud *et al.*, 2002;Toyoda *et al.*, 2010). We can exclude this possibility for the superoxide dismutase SodA, which showed a strong (26.6 fold) induction of the isoform with the more acid pI (spot 375). However, the mass spectrometry data suggest that this isoform likely had a higher level of tryptophan oxidation than the less acidic isoform (spot 376). Thus, acidic pI shift is a posttranslational effect of oxidative stress and in accordance with the lack of transcriptional changes for these enzymes (Table 3 and supplementary Table S6).

Conclusions

The delta subunit of the RNA polymerase, which is required for the transcriptional specificity, affects gene expression and physiological traits in *S. mutans* in a global way. By performing a proteome comparison, we demonstrated a significant impact of RpoE in *S. mutans*, but not a specific role for stress adaptation. We found that the Δ*rpoE* mutant had reduced abundance of proteins involved in carbohydrate metabolism and energy production under all conditions. Moreover, changes in the abundance of proteins required for stress response were observed in the mutant at log phase in the absence of stress. The relaxed transcriptional specificity caused by lack of RpoE may cause internal stress, thus reduce the overall fitness of the mutant. The mutant had weaker but similar changes as the wild type after adaptation to acid and peroxide stress for two hours. A comparison of proteome and transcriptome data suggests that at least half of the proteome changes are likely due to relaxed transcriptional regulation in the mutant. Thus RpoE affects the proteome of *S. mutans* in a general way, but it is not involved in specific stress adaptation.

Authors' contributions

XX carried out the proteome experiment and data analysis, and drafted the manuscript. JL gave detailed instruction of proteome experiment procedure and provided helpful suggestion of proteome data analysis. WW gave detailed instruction of proteome experiment procedure, participated in protein identification, as well as the manuscript revision. HS and IWD conceived the study, oversaw the execution of the whole experiment, and revised the manuscript critically with important comments. All authors revised and approved the final manuscript.

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Figure legends

Fig. 1. Principal component analysis of the total proteome data (280 spots) from 2-DE. The distance between samples on the plot is proportional to the variance in the abundance of their protein spots. Samples of the Δ*rpoE* mutant are shown in lighter colours. W: wild type; M: Δ*rpoE*; 0: log growth phase; 1: early stationary growth phase; 2: acid stress (pH 5); 3: H_2O_2 stress (2 mM H_2O_2); 4: acid/H_2O_2 (pH 5/2 mM H_2O_2) stress.

Fig. 2. 2-DE images of the proteome of *S. mutans* wild type and the Δ*rpoE* mutant. Upper and lower panels show the proteome of the wild type and the Δ*rpoE* mutant at early stationary phase, respectively. Gels were stained with Sypro-RuBPS fluorescent dye, then recorded with a CCD based Fujifilm LAS-1000 image analyzer with light spots on the black background. Images were inverted by Progenesis SameSpots software. Only half of the gel images with major changes are shown. Red arrows highlight the differentially expressed proteins with fold of changes >=2 ($P<0.05$) in the carbohydrate metabolism and energy production, all of which were down-regulated in the mutant in comparison to the wild type.

Fig. 3. Reduction of glycolysis enzymes in the Δ*rpoE* mutant under oxidative stress. Glycolysis enzymes were down-regulated in the mutant compared to the wild type under oxidative stress. The images of representative protein spots are shown on the left panel. On the right panel, enzymes involved in the glycolysis pathway are enclosed in ovals. Folds of abundance changes of the different proteins are given in brackets on the right panel.

Table 1. Proteins which showed significantly changed abundance in *S. mutans* Δ*rpoE* mutant in comparison to the wild type at log phase of growth and the corresponding changes in gene expression.

Spot No.	Gene code	Gene name	Protein name	M0/W0*	P (anova)	M0/W0 t§	P value
carbohydrate metabolism							
183	SMU.1233	*deoB*	phosphopentomutase	-1.9	8.5E-04	-1.5	6.0E-12
744	SMU.1247	*eno*	enolase	-1.8	4.7E-02	ns	ns
179	SMU.139	*oxdC*	putative oxalate decarboxylase	-1.7	4.4E-02	-6.1	7.0E-15
71	SMU.1077	*pgm*	phosphoglucomutase	-1.6	4.0E-03	1.7	2.9E-05
352	SMU.636	*nagB*	glucosamine-6-phosphate isomerase (N-acetylglucosamine-6-phosphate isomerase)	-1.6	3.7E-02	-2.2	7.9E-11
492	SMU.2038	*pttB*	phosphotransferase system, trehalose-specific IIBC component (EIIBC-tre)	1.4	2.5E-02	-1.2	9.5E-08
305	SMU.99	*fbaA*	fructose-1,6-biphosphate aldolase	1.5	1.0E-02	ns	ns
energy production and conversion							
235	SMU.127	*adhA*	acetoin dehydrogenase, E1 component alpha subunit	-2.5	2.0E-03	-3.6	2.1E-10
458	SMU.402	*pfl*	pyruvate formate-lyase	-1.7	2.5E-02	1.1	3.2E-03
676	SMU.402	*pfl*	pyruvate formate-lyase	-1.3	1.3E-02	1.1	3.2E-03
272	SMU.1043c	*eutD*	phosphotransacetylase	2.0	4.0E-03	1.5	1.2E-08
acid defense							
481	SMU.137	*mleS*	malolactic enzyme	-2.1	6.0E-03	-7.4	2.9E-15
237	SMU.233	*ilvC*	ketol-acid reductoisomerase	1.7	9.6E-04	ns	ns
497	SMU.2128	*ilvd*	dihydroxy-acid dehydratase	1.5	3.1E-02	1.3	1.5E-03
215	SMU.1203	*ilvE*	branched-chain amino acid	2.0	7.4E-04	1.2	4.9E-06

			aminotransferase IlvE				
166	SMU.364	*glnA*	glutamine synthetase type 1 (glutamate--ammonia ligase)	2.6	4.0E-03	1.1	2.5E-07
oxidative defense							
150	SMU.838	*gshR*	glutathione reductase	1.8	9.0E-03	1.0	2.4E-09
519	SMU.765	*nox1*	alkyl hydroperoxidase reductase, subunit F (NADH oxidase)	-1.7	1.7E-02	-1.1	6.5E-03
protein turnover							
389	SMU.1672	*clpP*	ATP-dependent Clp protease, proteolytic subunit	-2.3	1.0E-02	-1.4	6.3E-04
44	SMU.562	*clpE*	ATP-dependent protease ClpE	-1.6	8.7E-06	-1.7	7.0E-07
469	SMU.2036	*pepO*	endopeptidase O	-1.6	5.0E-03	-1.0	2.3E-04
558	SMU.466	*pepC*	cysteine aminopeptidase C	1.4	9.0E-03	-1.0	4.1E-07
translation							
63	SMU.2101	*asps*	aspartyl-tRNA synthetase	2.2	2.0E-03	1.2	5.8E-04
277	SMU.445	*glyQ*	glycyl-tRNA synthetase alpha subunit	2.1	1.0E-03	-2.1	3.5E-11
147	SMU.2102	*hiss*	histidyl-tRNA synthetase	1.5	1.8E-02	-1.4	7.2E-07
501	SMU.773c	*lysS*	lysyl-tRNA synthetase	1.3	2.6E-02	-1.2	7.8E-07
719	SMU.1847	*elp*	translation elongation factor P	-1.5	4.2E-02	-1.2	1.1E-04
lipid metabolism							
198	SMU.1739	*fabF*	3-oxoacyl-(acyl-carrier-protein) synthase	1.4	8.0E-03	-1.0	1.7E-02
638	SMU.1742c	*fabK*	trans-2-enoyl-ACP reductase II	1.7	1.0E-03	ns	ns
others							
94	SMU.475		conserved hypothetical protein	-3.4	4.0E-03	-1.0	6.0E-05
748	SMU.1760c		conserved hypothetical protein	-1.7	1.2E-05	1.2	3.6E-06

400	SMU.961	conserved hypothetical protein	-2.1	3.2E-02	-1.2	2.6E-13
72	SMU.1444c *yqgA*	metallo-beta-lactamase superfamily proteinhetical protein	-1.4	1.6E-02	-1.2	9.4E-08
641	SMU.1348c *psaA*	ABC transporter, ATP-binding protein	1.5	4.0E-03	ns	ns
214	SMU.1653 *serA*	D-3-phosphoglycerate dehydrogenase	1.7	9.0E-03	1.3	1.8E-06
408	SMU.1859 *ssb*	single-stranded DNA-binding protein	-1.8	3.0E-03	ns	ns
115	SMU.2157 *guaB*	inosine monophosphate dehydrogenase	1.8	1.0E-03	1.0	3.1E-05

528 * Protein fold of changes in the Δ*rpoE* mutant compared to the wild type at log phase (M0/W0).

529 § t: transcriptome data. Changes in gene expression were determined under the same
530 experimental conditions as for the proteome All fold of change values which were significant
531 (with p<0.005) are shown. Those with p>=0.005 are indicated as ‘‘ns’’ (not significant). See
532 methods for details on microarray methodology (Xue *et al.*, 2010).

533

Table 2. Proteins which showed significantly changed abundance in *S. mutans* Δ*rpoE* mutant in comparison to the wild type at early stationary phase of growth and the corresponding changes in gene expression.

Spot No.	Gene code	Gene name	Protein name	M1/W1*	P (anova)	M1/W1 t§	P value
carbohydrate metabolism							
74	SMU.883	*dexB*	glucan 1,6-alpha-glucosidase (dextran glucosidase DexB)	-3.4	4.6E-04	1.2	7.0E-11
71	SMU.1077	*pgm*	phosphoglucomutase	-3.1	9.2E-04	-1.5	2.9E-05
179	SMU.139	*oxdC*	putative oxalate decarboxylase	-2.6	2.2E-02	-8.4	7.0E-15
703	SMU.882	*msmK*	multiple sugar-binding ABC transporter, ATP-binding protein MsmK	-2.2	4.9E-02	1.2	1.8E-12
626	SMU.888	*galE*	UDP-galactose 4-epimerase GalE	-2.0	1.0E-02	1.8	2.1E-10
183	SMU.1233	*deoB*	phosphopentomutase	-2.0	4.0E-03	-1.7	6.0E-12
125	SMU.881	*gtfA*	sucrose phosphorylase GtfA	-1.9	4.6E-02	1.2	4.1E-12
750	SMU.360	*gapC*	glyceraldehyde-3-phosphate dehydrogenase	-1.6	5.0E-03	ns	ns
677	SMU.1564	*glgA*	glycogen phosphorylase	-1.4	1.5E-02	-1.6	4.1E-12
665	SMU.676	*gapN*	NADP-dependent non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase	1.3	3.3E-02	1.2	3.6E-04
energy production and conversion							
458	SMU.402	*pfl*	pyruvate formate-lyase	-4.0	4.9E-04	-1.5	3.2E-03
461	SMU.402	*pfl*	pyruvate formate-lyase	-2.8	4.0E-03	-1.5	3.2E-03
459	SMU.402	*pfl*	pyruvate formate-lyase	-3.0	2.0E-03	-1.5	3.2E-03
676	SMU.402	*pfl*	pyruvate formate-lyase	-2.6	6.0E-03	-1.5	3.2E-03

17	SMU.148	*adhE*	alcohol-acetaldehyde dehydrogenase	-2.6	1.4E-02	-2.5	1.2E-08
16	SMU.148	*adhE*	alcohol-acetaldehyde dehydrogenase	-2.1	2.5E-02	-2.5	1.2E-08
272	SMU.1043c	*eutD*	phosphotransacetylase	-1.4	3.9E-02	-1.3	1.2E-08
oxidative defense							
150	SMU.838	*gshR*	glutathione reductase	2.6	1.7E-02	2.3	2.4E-09
146	SMU.838	*gshR*	glutathione reductase	1.7	7.0E-03	2.3	2.4E-09
acid defense							
215	SMU.1203	*ilvE*	branched-chain amino acid aminotransferase IlvE	1.7	3.5E-02	-1.0	4.9E-06
238	SMU.233	*ilvC*	ketol-acid reductoisomerase	1.5	1.0E-02	ns	ns
protein turnover,chaperone							
389	SMU.1672	*clpP*	ATP-dependent Clp protease, proteolytic subunit	-2.5	3.9E-02	-1.5	6.3E-04
57	SMU.82	*dnaK*	chaperone protein (heat shock protein) DnaK (HSP-70)	1.6	4.0E-03	-1.1	2.9E-06
translation							
628	SMU.2032	*rpsB*	30S ribosomal protein S2	-2.0	2.0E-03	1.1	1.5E-06
581	SMU.1200	*rpsA*	ribosomal protein S1	-1.7	2.0E-03	ns	ns
others							
94	SMU.475		conserved hypothetical protein	-2.1	6.0E-03	1.2	6.0E-05
408	SMU.1859	*ssb*	single-stranded DNA-binding protein	-2.2	7.0E-03	ns	ns
748	SMU.1760c		conserved hypothetical protein	1.6	4.3E-02	1.8	3.6E-06
728	SMU.557	*divIVA*	cell division initiation protein DivIVA	-2.0	1.0E-02	ns	ns

268	SMU.496	*cysK*	cysteine synthetase A (O-acetylserine lyase)	-2.0	3.6E-02	-2.8	5.1E-07
108	SMU.2157	*guaB*	inosine monophosphate dehydrogenase	1.7	5.0E-03	1.1	3.1E-05

[*] Protein fold of changes in the Δ*rpoE* mutant compared to the wild type at early stationary phase (M1/W1).

[§] t: transcriptome data. Changes in gene expression were determined under the same experimental conditions as for the proteome. All fold of change values which were significant (with $p<0.005$) are shown. Those with $p>=0.005$ are indicated as ‘‘ns’’ (not significant). See Table 1 for further explanations.

544 **Table 3. Proteins which showed significantly changed abundance in *S. mutans* Δ*rpoE***

545 **mutant in comparison to the wild type under peroxide stress and the corresponding**

546 **changes in gene expression.**

Spot No.	Gene code	Gene name	Protein name	M3/W3*	P (anova)	M3/W3 t§	P value
carbohydrate transport and metabolism							
744	SMU.1247	*eno*	enolase	-3.0	8.0E-03	ns	ns
118	SMU.1190	*pykF*	pyruvate kinase	-2.7	4.0E-03	ns	ns
622	SMU.1191	*pfkA*	6-phosphofructokinase	-2.7	5.0E-03	-1.1	3.7E-02
440	SMU.360	*gapC*	glyceraldehyde-3-phosphate dehydrogenase	-2.1	3.3E-02	ns	ns
305	SMU.99	*fbaA*	fructose-1,6-biphosphate aldolase	-1.5	3.5E-02	ns	ns
70	SMU.1077	*pgm*	phosphoglucomutase	-2.3	1.3E-02	-2.1	2.9E-05
71	SMU.1077	*pgm*	phosphoglucomutase	-1.7	3.4E-02	-2.1	2.9E-05
179	SMU.139	*oxdC*	putative oxalate decarboxylase	-1.7	4.7E-02	-3.6	7.0E-15
677	SMU.1564	*glg*	glycogen phosphorylase	1.8	4.0E-02	-1.8	4.1E-12
703	SMU.882	*msmK*	multiple sugar-binding ABC transporter, ATP-binding protein MsmK	1.5	2.5E-02	1.3	1.8E-12
energy production and conversion							
252	SMU.1115	*ldh*	lactate dehydrogenase	-2.2	4.2E-02	ns	ns
216	SMU.1309c	*gldA-2*	glycerol dehydrogenase	1.7	6.0E-03	-1.0	9.2E-09
458	SMU.402	*pfl*	pyruvate formate-lyase	1.5	1.8E-02	-1.4	3.2E-03
459	SMU.402	*pfl*	pyruvate formate-lyase	1.4	3.8E-02	-1.4	3.2E-03
oxidative defense							

375	SMU.629	*sodA*	manganese-type superoxide dismutase, Fe/Mn-SOD	-8.1	5.0E-03	1.5	3.3E-04
519	SMU.765	*nox1*	alkyl hydroperoxidase reductase, subunit F (NADH oxidase)	-1.9	7.0E-03	1.5	6.5E-03
146	SMU.838	*gshR*	glutathione reductase	-1.4	5.2E-04	1.6	2.4E-09
protein turnover,chaperone							
469	SMU.2036	*pepO*	endopeptidase O (peptidase)	-1.9	1.2E-02	1.2	2.3E-04
149	SMU.466	*pepC*	cysteine aminopeptidase C	-1.6	2.6E-02	-1.1	4.1E-07
467	SMU.956	*clp*	ATP-dependent Clp protease, ATP-binding subunit	-1.5	4.0E-03	1.7	1.6E-10
466	SMU.956	*clp*	ATP-dependent Clp protease, ATP-binding subunit	-1.4	4.3E-02	1.7	1.6E-10
translation							
581	SMU.1200	*rpsA*	ribosomal protein S1	-2.1	1.1E-02	ns	ns
453	SMU.1510	*pheT*	phenylalanyl-tRNA synthetase beta subunit	-2.0	5.0E-02	-1.5	1.7E-09
147	SMU.2102	*hiss*	histidyl-tRNA synthetase (histidine--tRNA ligase)	1.5	2.0E-02	-1.1	7.2E-07
lipid metabolism							
326	SMU.1746c	*fabM*	enoyl-CoA hydratase/trans-2, cis-3-decenoyl-ACP isomerase	-2.3	2.0E-03	ns	ns
198	SMU.1739	*fabF*	3-oxoacyl-(acyl-carrier-protein) synthase	-1.7	2.3E-02	-1.1	1.7E-02
others							
624	SMU.1760c		conserved hypothetical protein	-1.9	3.0E-03	-1.0	3.6E-06
48	SMU.751		conserved hypothetical protein (possible transcriptional regulator)	1.5	9.0E-03	-1.4	1.4E-07
237	SMU.233	*ilvC*	ketol-acid reductoisomerase	-1.5	5.0E-02	ns	ns
726	SMU.557	*divIV*	cell division initiation protein	3.9	1.0E-02	ns	ns

A DivIVA

[*]Fold of changes of proteins in the Δ*rpoE* mutant (M3) compared to the wild type (W3) under peroxide stress.

[§] t: transcriptome data. Changes in gene expression were determined under the same conditions as those for the proteome. All transcriptome data which were significant (with $p<0.005$) are shown. Those with $p>=0.005$ are indicated as ‘‘ns’’ (not significant).

552 **Table 4. Correlation between proteome and transcriptome data.**

	M0/W0*	M1/W1	M2/W2	M3/W3	M4/W4
N#	37	30	29	29	29
correlation (%)‖	46	50	55	30	52
inverse relationship?	19	33	14	30	10
no sign. Changes in transcr.(%)	35	17	31	40	38

553 *W: wild type, M: mutant. 0: log phase, 1: early stationary phase, 2: acid stress, 3: peroxide stress,

554 4: combined acid/peroxide stress.

555 #number of regulated proteins.

556 ‖gene expression changes in the same direction as protein abundance changes

557 ?gene expression changes in the opposite direction as protein abundance changes

Additional files

Figure S1. Growth and viability of *S. mutans* wild type and the Δ*rpoE* mutant under the experimental conditions.

Table S1. MALDI-TOF identification of proteins in *S. mutans*.

Table S2. Proteins which showed significantly changed abundance in *S. mutans* Δ*rpoE* mutant in comparison to the wild type under acid stress and the corresponding changes in gene expression.

Table S3. Proteins which showed significantly changed abundance in *S. mutans* Δ*rpoE* mutant in comparison to the wild type under acid and peroxide combined stress and the corresponding changes in gene expression.

Table S4. Proteins with significantly changed abundance at early stationary growth phase in comparison to log phase in *S. mutans* wild type and the Δ*rpoE* mutant, and the corresponding changes in gene expression.

Table S5. Proteins with significantly changed abundance under acid stress in comparison to log phase in *S. mutans* wild type and the Δ*rpoE* mutant, and the corresponding changes in gene expression.

Table S6. Proteins with significantly changed abundance under peroxide stress in comparison to log phase in *S. mutans* wild type and the Δ*rpoE* mutant, and the corresponding changes in gene expression.

Table S7. Proteins with significantly changed abundance under acid and oxidative combined stress in comparison to log phase in *S. mutans* wild type and the Δ*rpoE* mutant, and the corresponding changes in gene expression.

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