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Global Analysis of Heat Shock Response in *Desulfovibrio vulgaris* Hildenborough

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Desulfovibrio vulgaris **Hildenborough belongs to a class of sulfate-reducing bacteria (SRB) and is found ubiquitously in nature. Given the importance of SRB-mediated reduction for bioremediation of metal ion contaminants, ongoing research on *D. vulgaris* has been in the direction of elucidating regulatory mechanisms for this organism under a variety of stress conditions. This work presents a global view of this organism's response to elevated growth temperature using whole-cell transcriptomics and proteomics tools. Transcriptional response (1.7-fold change or greater; $Z \geq 1.5$) ranged from 1,135 genes at 15 min to 1,463 genes at 120 min for a temperature up-shift of 13°C from a growth temperature of 37°C for this organism and suggested both direct and indirect modes of heat sensing. Clusters of orthologous group categories that were significantly affected included posttranslational modifications; protein turnover and chaperones (up-regulated); energy production and conversion (down-regulated), nucleotide transport, metabolism (down-regulated), and translation; ribosomal structure; and biogenesis (down-regulated). Analysis of the genome sequence revealed the presence of features of both negative and positive regulation which included the CIRCE element and promoter sequences corresponding to the alternate sigma factors σ^{32} and σ^{54}. While mechanisms of heat shock control for some genes appeared to coincide with those established for *Escherichia coli* and *Bacillus subtilis*, the presence of unique control schemes for several other genes was also evident. Analysis of protein expression levels using differential in-gel electrophoresis suggested good agreement with transcriptional profiles of several heat shock proteins, including DnaK (DVU0811), HtpG (DVU2643), HtrA (DVU1468), and AhpC (DVU2247). The proteomics study also suggested the possibility of posttranslational modifications in the chaperones DnaK, AhpC, GroES (DVU1977), and GroEL (DVU1976) and also several periplasmic ABC transporters.**

Sulfate-reducing bacteria (SRB) represent a class of organisms characterized by nonphotosynthetic generation of energy through electron transfer-coupled phosphorylation using sulfate as the terminal electron acceptor, and thus they play a critical role in sulfur cycling (19). Aside from a role for these bacteria in biocorrosion, leading to problems in the petroleum industry, recent interest in SRB has grown due to their potential for bioremediation of toxic metals (24). *Desulfovibrio vulgaris* Hildenborough, a member of the δ-proteobacteria, is a model SRB that has been extensively studied over the past few years (11, 13, 15, 17, 18, 21, 28, 41), although its responses to conditions of heat stress have not yet been reported. Perturbation experiments such as temperature up-shift and other stressors can be applied to identify genes which might affect the rate of metal reduction by the SRB and may be used to build models that describe the process of bioremediation.

The recently released genome sequence of this organism (19) revealed the presence of a large number of regulatory elements corresponding to some widely conserved regulons among eubacteria and others specific to the δ-proteobacteria (31). A change in ambient temperature is a common stress condition experienced by free-living organisms; the response to heat shock represents a protective and homeostatic response to counteract temperature-induced damage in cells. The ubiquitous response to this stress condition is marked by the large-scale induction of heat shock proteins (HSPs) which include molecular chaperones that assist folding nascent proteins and repairing damaged proteins and ATP-dependent proteases that degrade misfolded proteins. *Escherichia coli* and *Bacillus subtilis* have long served as paradigms for heat shock response in gram-negative and gram-positive bacteria (2, 35). Heat shock studies in these model bacteria have led to the identification of a variety of DNA elements (like CIRCE, for "controlled inverted repeat of chaperone expression," HAIR, for "HspR-associated inverted repeat," and ROSE, for "repression of heat shock gene expression") and corresponding transcriptional regulators (HrcA, HspR, and CtsR, besides alternate sigma factors) during negative or positive heat shock regulation in bacteria (26). The genome sequence of *D. vulgaris* reveals features of negative heat shock regulation, as observed in *B. subtilis* (CIRCE and HrcA), and those of positive regulation, like those in *E. coli* (σ^{32} and σ^{54}), and thus represents an interesting blend of gram-positive and gram-negative heat shock-regulatory elements (26). In the present study, we used tools for quantifying transcriptome and proteome levels in this SRB to add to the growing body of heat shock studies in bacterial systems.

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MATERIALS AND METHODS

The stress experiment. Glycerol stocks of *D. vulgaris* Hildenborough stored at −80°C were used to grow three cultures (100 ml each) anaerobically on lactate-sulfate medium (29) at 37°C up to an optical density at 600 nm (OD_{600}) of 0.6. Each was then used to inoculate one of three larger cultures (1,440 ml each) in a 2-liter glass bottle that was grown under similar conditions to early log phase (OD_{600} = 0.3). From each bottle, 60-ml aliquots were then transferred anaerobically to 24 sealed nitrogen-filled culture bottles (166 ml). The three biological replicates were treated separately for analysis of the transcriptome such that four sealed culture bottles per biological replicate per time point were obtained. For each biological replicate, two sealed culture bottles were transferred to a shaker-water bath maintained at 50°C for the following time intervals: 15, 30, 60, 90, and 120 min. Time intervals were chosen taking into account the slow growth rate of *D. vulgaris* (5). The remaining two bottles were maintained at 37°C at the time intervals described above. The average cell density for cultures at 50°C (OD_{600} = 0.54) was only slightly different from that of the 37°C cultures (OD_{600} = 0.56) after 120 min of treatment, indicating that the shocked culture did not lyse at this temperature. At the end of each time interval, contents of the control and treatment cultures were transferred to 50-ml prechilled (4°C) Falcon tubes and spun at 10,000 × *g* for 10 min at 4°C. The resulting supernatant was discarded, and the cell pellets were stored at −80°C until total RNA extraction.

Cells for proteomic analysis were grown in a separate experiment. Cultures (60 ml) in six sealed bottles (166 ml) were grown at 37°C to an OD_{600} of 0.3 as detailed above. Three culture bottles were placed in a 50°C water bath for 60 min, while the remaining three were maintained at 37°C. Cells were harvested as described above, with the exception that cell pellets were washed once with 100 mM Tris-HCl, pH 8.5, and collected again by centrifugation before storage at −80°C.

Total RNA, genomic DNA, and soluble protein extraction and labeling. Total cellular RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA) per the manufacturer's instructions. RNA extracts were purified using the RNeasy Mini kit (QIAGEN, Valencia, CA), and on-column DNase digestion was performed with the RNase-free DNase set (QIAGEN, Valencia, CA) to remove genomic DNA contamination according to the manufacturer's procedure. cDNA probes were generated from 10 μg of purified total RNA using reverse transcriptase and then labeled as described previously (40). Random hexamers (Invitrogen) were used for priming, and the fluorophore Cy5-dUTP (Amersham Biosciences, Piscataway, NJ) was used for labeling. After the labeling, RNA was removed by NaOH treatment and cDNA was immediately purified with a QIAGEN PCR Mini kit. Genomic DNA was extracted from *D. vulgaris* cultures at stationary phase using a standard protocol detailed elsewhere (49) and labeled with the fluorophore Cy3-dUTP (Amersham Biosciences, Piscataway, NJ). The efficiency of labeling was routinely monitored by measuring the absorbance at 260 nm (for DNA concentration), 550 nm (for Cy3), or 650 nm (for Cy5).

For soluble protein extraction, frozen cell pellets from stressed and unstressed cells were resuspended in 100 mM Tris-HCl, pH 8.5 (2 ml), and lysed using a sonic dismembrator (Model #550; Fisher Scientific, Pittsburgh, PA). The cell lysate was centrifuged at 20,000 × *g* for 15 min at 4°C to separate the cell debris. The supernatant was subsequently used to determine the soluble protein profile of the cell. Protein samples were always maintained below 4°C, and a protease inhibitor cocktail consisting of EDTA-Na_2 (0.5 mM), pepstatin (10 μM), bestatin (0.13 mM), and Pefabloc SC plus (Roche Applied Science, Indianapolis, IN) (0.4 mM) was added to minimize degradation. Protein concentrations were determined using the bicinchoninic acid protein assay (Pierce, Rockford, IL) with bovine serum albumin as the standard. Samples obtained from replicate cultures were pooled and sent to Integrated Biotechnology Laboratories, Athens, GA, for processing and fluorescence two-dimensional (2-D) difference gel electrophoresis, described in brief here. Proteins obtained from stressed and nonstressed cultures were resuspended in 8 M urea, 4% (wt/vol) 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS), 15 mM Tris, pH 8.3. Samples were then centrifuged at 60,000 × *g* for 4 h at 20°C to remove insoluble proteins and cell debris. Supernatants were concentrated with three buffer exchanges of the urea-CHAPS buffer using Microcon YM-10 centrifugal filter devices (Millipore). A 50-μg sample of each extract was labeled with 200 pmol CyDye DIGE Fluor minimal dye Cy3 or Cy5 (Amersham Biosciences, Piscataway, NJ). A pooled protein extract (50 μg) was labeled with CyDye DIGE Fluor Cy2 minimal dye (Amersham Biosciences, Piscataway, NJ). The labeling reaction, controlled to label one lysine per protein, was carried out at 4°C for 30 min and quenched with 10 nmol lysine.

Whole-genome DNA microarray construction. DNA microarrays covering 3,482 of the 3,531 protein-coding sequences of the *D. vulgaris* genome were constructed with 70mer oligonucleotide probes (19). Optimal probe design was achieved using previously developed computer software tools (7, 34, 43), and synthesized oligonucleotide probes were spotted onto UltraGAPS glass slides (Corning Life Sciences, Corning, NY) using a BioRobotics Microgrid II microarrayer (Genomic Solutions, Ann Arbor, MI) as described elsewhere (40).

Microarray hybridization, washing, and scanning. To hybridize a single glass slide, the Cy5-dUTP-labeled cDNA probes obtained from stressed or unstressed cultures were mixed in equal amounts with the Cy3-dUTP-labeled genomic DNA (39, 46). To accomplish this, dried probes were mixed and resuspended in 35 to 40 μl of hybridization solution that contained 50% (vol/vol) formamide, 5× saline-sodium citrate (SSC; 1× SSC is 0.15 M NaCl plus 0.015 M Na citrate, pH 7.0), 0.1% (wt/vol) sodium dodecyl sulfate (SDS), and 0.1 mg herring sperm DNA (Invitrogen)/ml. The hybridization solution was incubated at 95 to 98°C for 5 min, centrifuged briefly to collect condensation, kept at 50°C, and applied onto microarray slides. Hybridization was carried out in hybridization chambers (Corning Life Sciences, Corning, NY) at 45°C overnight (16 to 20 h). Ten microliters of 3× SSC solution was added to the wells at the ends of the microarray slides to maintain proper chamber humidity and probe hydration around the edges of the coverslip. Microarray slides were washed according to the instructions for spotted oligonucleotide microarrays on UltraGAPS slides by the manufacturer (Corning) in the following steps: twice in a solution containing 2× SSC and 0.1% (wt/vol) SDS at 42°C for 5-min periods, twice for 10 min each in a solution containing 0.1× SSC and 0.1% (wt/vol) SDS at room temperature, and twice for 1-min periods in 0.1× SSC at room temperature. After drying under a stream of N_2, the slides were scanned for the fluorescent intensity of both the Cy5 and Cy3 fluorophores using the ScanArray Express microarray analysis system (Perkin Elmer). To determine signal fluorescence intensities for each spot, 16-bit TIFF scanned images were analyzed with the software ImaGene version 6.0 (Biodiscovery, Marina Del Rey, CA) that quantifies spot signal, spot quality, and background fluorescent intensities. Any spot that had fewer than 75% of pixels or more than 3 standard deviations above the local background in both channels was rejected (14). Note that each gene was represented by duplicate spots on a single microarray slide. For a given time point (control or treatment), three microarray slides corresponding to three biological replicates were used. To estimate the average log (base 2) expression levels from replicate arrays, log expression levels from each array were normalized globally. This was calculated as follows. First, the net signal of each spot was calculated by subtracting out the background and adding a pseudosignal of 100 to enforce a positive value. If the resulting net signal was still less than 50, a value of 50 was used. Next, the expression level for each spot was calculated from the ratio of the two channels (mRNA over genomic DNA). Expression levels for each replicate were normalized such that the total expression level over the spots that were present on all replicates was identical. Finally, the mean expression level and standard deviation of each spot were estimated, requiring $n > 1$, where n is the number of scorable replicates. To estimate the differential gene expression between the control and treatment conditions, normalized log ratios were calculated. Each log ratio was calculated as $\log_2(\text{treatment}) - \log_2(\text{control})$. This log ratio was normalized using LOWESS (locally weighted scatterplot smoothing) on the difference versus the sum of the log expression level (12). Since sector-based artifacts were detected, each log ratio was further normalized by subtracting the median of all spots within each sector. The final normalized log ratio ($\log_2 R$) was calculated from the average ratio of spots for each gene. To assess the significance of the normalized log ratio, a Z score was calculated by the following equation (0.25 is a pseudovariance term): $Z = \log_2(\text{treatment}/\text{control})/\sqrt{(0.25 + \Sigma_{\text{variance}})}$.

Reported log ratios in this work are for genes with $Z \geq 1.5$ (absolute value).

Differential in-gel electrophoresis (DIGE) and data analyses. DIGE analysis was performed at the Integrated Biotechnology Laboratories as follows. Labeled protein extracts, control, experimental, and pooled samples, were combined, and proteins were separated on pH 3 to 10 IPG (immobilized pH gradient) strips in 8 M urea, 2% (wt/vol) CHAPS, 0.5% (wt/vol) IPG buffer, 18.2 mM dithiothreitol (DTT), and 0.002% (wt/vol) bromophenol blue using active rehydration at 30 V for 12 h for a total of 32,000 V · h (IPGphor Isoelectric Focusing System). IPG strips were subsequently reduced in 6 M urea, 2% (wt/vol) SDS, 30% (vol/vol) glycerol, 50 mM Tris, pH 8.8, and 0.002% (wt/vol) bromophenol blue with 64.8 mM DTT for 15 min at room temperature and then alkylated in the above buffer, replacing DTT with 135 mM iodoacetamide, for 15 min at room temperature. Proteins were then separated on SDS-polyacrylamide (8 to 15% [wt/vol]) gels. Three gels were run. Gels were fixed in 30% (vol/vol) ethanol and 7.5% (vol/vol) acetic acid overnight at room temperature. Gels were imaged using a Typhoon 9400 (Amersham Biosciences, Piscataway, NJ) and analyzed for twofold or greater differences in the volume ratio using DeCyder software (Amersham Biosciences, Piscataway, NJ). The volume ratio was calculated as the ratio of logarithmic values of the standardized volumes. Standardized volumes were

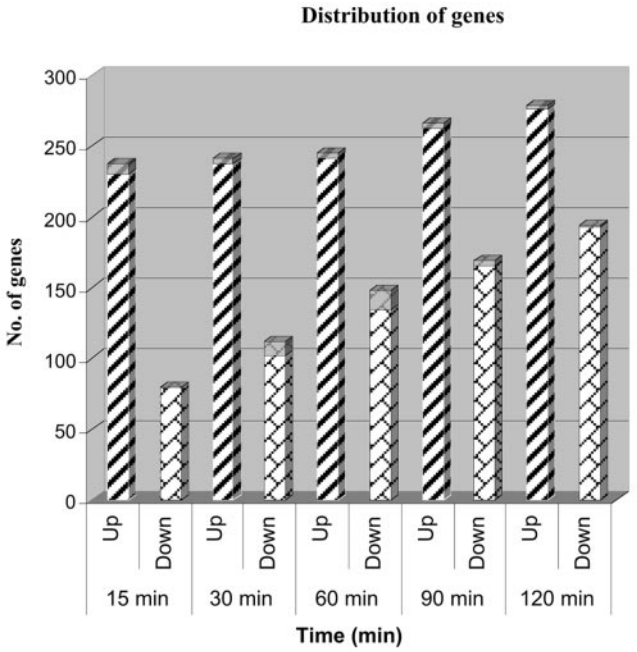

FIG. 1. Distribution of up- and down-regulated genes in *D. vulgaris* Hildenborough as a function of time upon a temperature up-shift of 13°C. Only those genes with $Z \geq 1.5$ (absolute value) and $\log_2 R \leq -2$ or $\log_2 R \geq 2$ were included in the plot. The gray region at the top of each data bar corresponds to genes from the megaplasmid. Up-regulated, $\log_2 R \geq 2$; down-regulated, $\log_2 R \leq -2$.

estimated from the ratio of spot volumes (intensity times the area of the spot) for the treatment (Cy5) or control (Cy3) sample to the spot volume of the pooled sample (Cy2). Gels were stained with Sypro Ruby (Invitrogen, Carlsbad, CA), destained in 10% (vol/vol) methanol and 6% (vol/vol) acetic acid for 30 min at room temperature, imaged, and matched to the Cy images with DeCyder software.

The pick list was created based on the Sypro image. With the Spot Handling Workstation (Amersham Biosciences, Piscataway, NJ), gel plugs of 2 mm in diameter were picked and washed, proteins were digested in-gel with trypsin, and the resulting peptides were extracted and dried. Briefly, plugs were washed twice with 50 mM ammonium bicarbonate–50% (vol/vol) methanol for 20 min at room temperature and once with 75% (vol/vol) acetonitrile for 20 min at room temperature and were dried at 40°C for 10 min. Dried plugs were incubated with 140 ng sequencing-grade trypsin (Promega, Madison, WI) at 37°C for 1 h. Peptides were extracted twice with 50% (vol/vol) acetonitrile–0.1% (wt/vol) TFA (trifluoroacetic acid) for 20 min at room temperature. Extracted peptides were spotted using partially saturated α-cyano-4-hydroxy-cinnamic acid (Sigma-Aldrich, St. Louis, MO), and mass spectrometry (MS) data were collected from a DE PRO Voyager (Applied Biosystems, Foster City, CA). Peptide MS data were searched against the *D. vulgaris* protein database (genome sequence derived [1] with the MASCOT search engine [Matrix Science, Boston, MA]) to identify the corresponding proteins using a MOWSE (molecular weight search) score cutoff of 60 (27).

RESULTS AND DISCUSSION

Transcriptional response: global view. *D. vulgaris* Hildenborough cultures in early logarithmic growth phase at 37°C were subjected to a temperature shift to 50°C. Global analyses of the changes in transcription were then performed. The number of genes being transcribed with a fold change of 1.7 or higher and corresponding to a Z score (absolute value) of 1.5 or greater varied from 1,135 genes at 15 min to 1,463 genes at 120 min (Table S1 in the supplemental material). This included 21 megaplasmid genes at 15 min and 11 at 120 min. The number of genes differentially expressed between controls and the heat-treated cells with $\log_2 R \leq -2$ or $\log_2 R \geq 2$ is plotted in Fig. 1. At early time points, exposure to elevated temperatures would likely reveal features of the heat shock paradigm observed in several bacteria. At later time points, however, cells would be either in stationary phase or moribund due to prolonged exposure, resulting in a more complex response pattern. The observed change in the number of up-regulated genes between 15 and 120 min increased by only 17% (238 genes at 15 min versus 279 genes at 120 min), whereas a much larger number of genes was down-regulated in that timeframe (81 versus 195 genes). We interpret these changes to signal that at later time points cellular survival was increasingly restricted to essential genes. Of the 3,395 chromosomal genes in *D. vulgaris*, 2,315 genes have so far been classified into one of the 20 COG (clusters of orthologous groups) functional categories (19). Table 1 displays COG functional categories for genes unequally impacted after 15 min of heat exposure. Table 2 displays expression ratios for SRB "Signature" genes from *D. vulgaris.* "Signature" genes, considered to be characteristic of the SRB either because of their dissimilatory sulfate metabolism or by sequence divergence of the genes, were identified by homology searches on available genome sequences from four sulfate-reducing microbes, *D. vulgaris*, *D. alaskensis* G20 (formerly *D. desulfuricans* G20), *Desulfotalea psychrophila*, and *Archaeoglobus fulgidus*, and compared to 209 sequenced bacterial genomes (E. J. Alm and A. P. Arkin, unpublished data). These include some genes involved in the dissimilatory sulfate reduction pathway and several oxidoreductases, for instance, genes from the operons *dsrA-cobB-1* (dissimilatory sulfite reductase), *apsB-qmoC* (adenylylsulfate reductase and quinone-interacting oxidoreductase), and rbO-roO (desulfoferrodoxin and rubredoxin-oxygen oxidoreductase) and others. Of the 46 SRB "Signature" genes, reproducible signals were obtained for 34 genes, the majority of which (27 genes) remained down-regulated during heat shock. The "Signature" genes, *apsA* (DVU0847), *apsB* (DVU0846), *dsrA* (DVU0402), and

TABLE 1. Distribution of up- and down-regulated genes (1.7-fold change or greater; $Z \geq 1.5$ [absolute value]) in *D. vulgaris* Hildenborough at 15 min upon a temperature up-shift of 13°C based on COG functional categories[a]

COG functional category	No. of genes			Major trend	*P* value
	Total	Up-regulated	Down-regulated		
Posttranslational modification, protein turnover, chaperones	94	34	9	Up	1.45E-04
Energy production and conversion	210	27	55	Down	3.76E-05
Nucleotide transport and metabolism	56	3	16	Down	3.76E-02
Translation, ribosomal structure, and biogenesis	153	12	56	Down	3.69E-11

[a] Major trends in each role category were determined using Fisher's exact test and are shown for $P < 0.05$ (false discovery rate method).

TABLE 2. Expression ratios (log_2R) for SRB "Signature" genes from *D. vulgaris*[a]

DVU no.	Description	Log_2R
DVU0105	Glutamine ABC transporter, ATP-binding protein, *glnQ*	-1.01^{60}
DVU0162	Carbamoyl-phosphate synthase, large subunit, *carB*	0.99^{30}
DVU0305	Ferredoxin II, *fdII*	−1.99
DVU0386	Amino acid ABC transporter, periplasmic amino acid-binding protein, *glnH*	−2.03
DVU0402	Dissimilatory sulfite reductase alpha subunit, *dsrA*	−3.28
DVU0403	Dissimilatory sulfite reductase beta subunit, *dvsB*	−2.59
DVU0405	Cobyrinic acid a,c-diamide synthase, *cobB-1*	1.92
DVU0456	DHH family protein	1.46
DVU0846	Adenylylsulphate reductase, beta subunit, *apsB*	−2.11
DVU0847	Adenylylsulphate reductase, alpha subunit, *apsA*	−1.71
DVU0849	Quinone-interacting membrane-bound oxidoreductase, *qmoB*	-1.28^{90}
DVU0850	Quinone-interacting membrane-bound oxidoreductase, *qmoC*	-1.11^{30}
DVU1067	Membrane protein, Bmp family	−1.21
DVU1069	Branched-chain amino acid ABC transporter, permease protein	−0.87
DVU1070	Branched-chain amino acid ABC transporter, ATP-binding protein, *rbsA*	-0.98^{30}
DVU1286	Integral membrane protein, *dsrP*	-1.28^{60}
DVU1287	Periplasmic (Tal), binds 2[4Fe-4S], *dsrO*	−1.08
DVU1288	Periplasmic (Sec) triheme cytochrome *c*, *dsrJ*	−1.14
DVU1289	Cytoplasmic, binds 2 [4Fe-4S], *dsrK*	−1.54
DVU1290	Inner membrane protein binds 2 heme b, *dsrM*	−1.25
DVU1568	Ferritin, *ftn*	-1.93^{30}
DVU1636	Inorganic pyrophosphatase, manganese dependent, *ppaC*	-1.69^{60}
DVU1867	Diaminopimelate epimerase, *dapF*	−1.18
DVU2108	MTH1175-like domain family protein	-1.41^{60}
DVU2310	Metallo-beta-lactamase family protein	2.9
DVU2735	Phenylacetate-coenzyme A ligase, *paaK-3*	−1.25
DVU2776	Dissimilatory sulfite reductase, gamma subunit, *dsrC*	-2.00^{60}
DVU2825	Pyruvate formate-lyase 1 activating enzyme, putative	1.04^{90}
DVU3183	Desulfoferrodoxin, *rbO*	-2.09^{120}
DVU3184	Rubredoxin	−1.53
DVU3185	Rubredoxin-oxygen oxidoreductase, *roO*	1.28^{30}
DVU3212	Pyridine nucleotide-disulfide oxidoreductase, *nox*	-0.82^{120}
DVU3290	Conserved domain protein	1.48^{60}
DVU3379	Ribonucleoside-diphosphate reductase	−1.18
DVU0848	Quinone-interacting membrane-bound oxidoreductase, *qmoA*	ND
DVU0883	Glutaredoxin, putative, *nrdH*	ND
DVU0884	Conserved hypothetical protein, *ftrB*	ND
DVU0951	Molybdopterin biosynthesis MoeA protein, putative, *moeA-2*	ND
ORF00713	Branched-chain amino acid ABC transporter, permease protein	ND
DVU2103	Iron-sulfur cluster-binding/ATPase domain protein	ND
DVU2271	Pyruvate formate-lyase activating enzyme, putative, *pflA*	ND
ORF02867	Amino acid ABC transporter, ATP-binding protein, *glnQ*	ND
DVU2422	Nitroreductase family protein	ND
DVU2990	Molybdopterin biosynthesis MoeA protein, *moeA*	ND
DVU3113	Carbamoyl-phosphate synthase, small subunit, *carA*	ND
DVU3276	Ferredoxin I	ND

[a] Data are shown for $Z \geq 1.5$ (absolute value) at 15 min unless otherwise indicated. For genes with $Z < 1.5$ (absolute value) at 15 min, data are shown for a different time point (appears as a superscript). ND, data not available at any time point.

dvsB (DVU0403), belong to the COG category of "energy production and conversion." Hence, it can be inferred (from Tables 1 and 2) that energy generation by sulfate reduction was down-regulated during heat shock.

Mechanisms of heat shock induction fall into two fundamentally different regulatory types, direct and indirect heat sensing (35). Expression of genes belonging to the former category continues at a high rate as long as cells are exposed to the high temperature, whereas genes from the latter category are induced transiently and the regulatory mechanism ensures return to prestimulus levels even if cells are kept longer at the high temperature (35). Temporal gene expression profiles of the up-regulated genes fall into three categories, those that increase in expression levels through the 120 min (direct heat sensing), those that decrease in expression levels from an initially high level (indirect heat sensing), and those with time-independent expression levels (Fig. 2). A majority of the commonly known HSPs were observed in the second (indirect heat sensing) grouping. These included the chaperones DnaK (DVU0811) and DnaJ (DVU3243); ATP-dependent proteases La (DVU1337), Clp (DVU1874), and membrane-associated zinc metalloprotease (DVU0865); peptidases DVU2494 and DVU1430; transcriptional regulators; heat-inducible transcription repressor HrcA (DVU0813); and sigma 54-dependent transcriptional regulators DVU0744 and DVU3305. The aforementioned genes were highly up-regulated at 15 min at 50°C, followed by a gradual decrease in the expression levels through 120 min. Although the gene encoding the ubiquitous heat shock chaperone DnaK (DVU0811) remained up-regulated during the heat exposure, there was a 3.2-fold difference

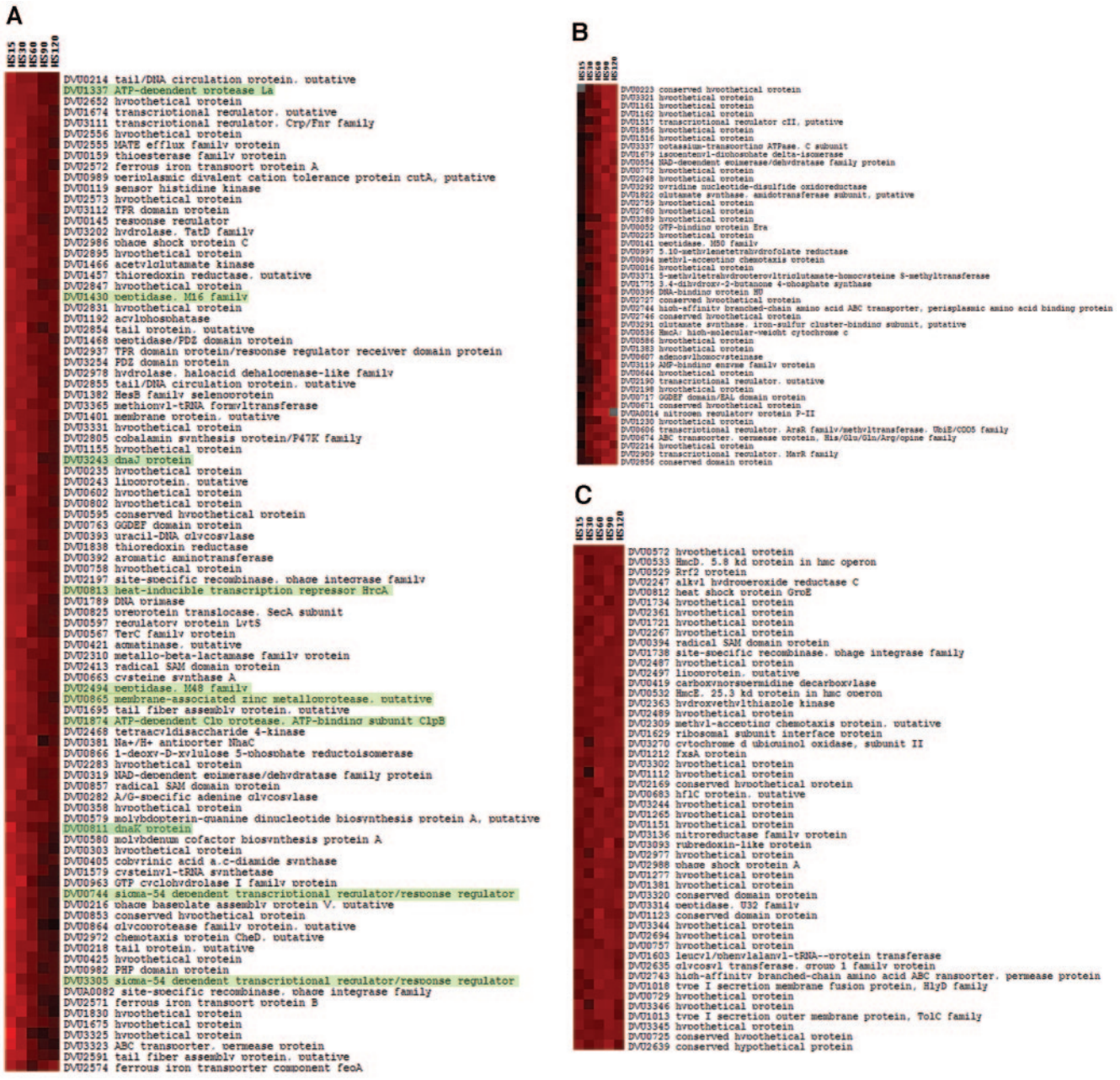

FIG. 2. Direct and indirect sensing of temperature upshift and time-independent expression profiles. K-means clustering of up-regulated genes in *D. vulgaris* Hildenborough from 15 min to 120 min upon a temperature upshift of 13°C. Only those genes with $Z \geq 1.5$ (absolute value) at all time points and at least one time point meeting the criterion of $\log_2 R \leq -2$ or $\log_2 R \geq 2$ were included in the clustering analysis. Commonly known heat shock proteins in the indirect heat-sensing grouping are highlighted in green.

in its expression level at 15 ($\log_2 R = 2.94$; $Z = 3.97$) versus 120 min ($\log_2 R = 1.26$; $Z = 2.02$). In *D. vulgaris*, regulation of *dnaK* might be governed by an indirect sensing mechanism involving HrcA and GroE. The GroE chaperonin system identified in *Bacillus subtilis* and other bacteria acts as a thermometer of heat stress and modulates the activity of the HrcA repressor by sensing nonnative proteins (25, 45). Further studies will be needed to identify mechanisms by which transcript levels for other genes in this category decrease with time.

Transcriptional response: transcriptional regulators. As mentioned previously, the genome sequence of *D. vulgaris* reveals features of negative heat shock regulation, as observed in *B. subtilis*, and those of positive regulation, as observed in *E. coli*. Heat shock regulation in *E. coli* is based on the use of alternate sigma factors, σ^{32} (RpoH), σ^{24} (RpoE), and σ^{54} (RpoN), that direct RNA polymerase to heat shock gene promoters and govern the expression of specific regulons (2, 22, 23, 30, 37, 44, 48).

TABLE 3. Computational predictions of σ^{32} (31) and σ^{54} promoters and CIRCE sites in the *D. vulgaris* genome[a]

Promoter or site and DVU no.	Description	Site	Start	Log_2R
σ^{32}-dependent promoters predicted in the *D. vulgaris* genome				
DVU1003	*dnaJ* domain protein	gaTGAAt-[N15]-CCCCtT	−114	ND
DVU1334	Trigger factor, *tig*	gTTGttg-[N15]-CCCCgT	−196	−1.43
DVU1584	Sigma 70 family protein, *rpoH*	aTTGAAA-[N12]-aaCTaT	−110	ND
DVU1977	Chaperonin, 10 kDa, *groES*	CaTaAAA-[N12]-CCCCtT	−239	ND
CIRCE sites predicted in the *D. vulgaris* genome				
DVU0813	Heat-inducible transcription repressor, *hrcA*	GGTGCTGTC-[N9]-GAGTGCAAC	−216	2.68
DVU1977	Chaperonin, 10 kDa, *groES*	cTgGCACTC-[N9]-GAGTGCcAA	−68	ND
σ^{54}-dependent promoters predicted in the *D. vulgaris* genome				
DVU0036	Hypothetical protein	GTGGCACGCTATCTGCT	−123	−5.39
DVU0102	Callon ABC transporter, periplasmic binding protein, *cckA*	GTGGCACGCCGGTCGCG	−102	1.09
DVU0139	Sensor histidine kinase	ATGGTACGCTGTTTGCT	−223	-0.88^{90}
DVU0151	HAMP domain/sigma 54 interaction domain protein	TTGGCACGGCCATTGCT	−160	1.45
DVU0162	Carbamoyl-phosphate synthase, large subunit, *carB*	ATGGTAGGGAGATTGCT	−125	0.99^{30}
DVU0163	Lipoprotein, putative	ATGGTAGGGAGATTGCT	−38	1.34^{90}
DVU0307	Flagella basal body rod domain protein	CTGGCACGGCTCGTGCT	−63	-1.00^{60}
DVU0320	Conserved hypothetical protein	ATTGCACGCTTCTTGCT	−248	1.8
DVU0358	Hypothetical protein	ATGGCATATGAATTGCT	−128	2.04
DVU0360	Acetolactate synthase, large subunit, biosynthetic type, *ilvB-1*	CCGGCAGCCCGCTTGCT	−83	1.27
DVU0367	Ser/Thr protein phosphatase family protein	ATGGCACCGGCGTCGCG	−231	0.90^{120}
DVU0406	Membrane protein, putative	TTGGCAACGTGGTTGCC	−12	1.62^{60}
DVU0407	Rare lipoprotein A family protein, *rlpA*	TTGGCAACGTGGTTGCC	−157	−1.4
DVU0571	Alanine dehydrogenase, *old*	TTGGCAAGGCCTTTGCT	−83	−1.3
DVU0631	Conserved hypothetical protein	ATGGCATGACTGTTTCG	−64	-1.20^{30}
DVU0682	DNA-binding protein, putative	GTGGCCCGATTTTTGCT	−130	0.90^{30}
DVU0725	Conserved hypothetical protein	GTCGCACATCCATTGCA	−236	2.51
DVU0731	Hypothetical protein	CTGGGGCGCCGGTTGCT	−78	-1.11^{120}
DVU0732	Vatyl-tRNA synthetase, *valS*	CTGGGGCGCCGGTTGCT	−14	−0.96
DVU0759	Peptidase, M29 family	AGGGCACTTGTCTTGCA	−28	3.78
DVU0841	Aspartate aminotransferase, putative	CTGGCGCGCCCCTTCCA	−64	−2.13
DVU0910	Flagellar motor switch protein, *fliM*	CTGGCCCCTATCTTGCA	−9	3.43
DVU0913	Conserved hypothetical protein	ACGGCCCGTTCCTTGCT	−1	-1.39^{60}
DVU0994	Hypothetical protein	TTGGCACTGGAGTTGTA	−68	1.98
DVU0995	ThlJ/Pfpl family protein	TTGGCACTGGAGTTGTA	−172	-0.89^{90}
DVU1062	Conserved hypothetical protein	ACGGCAAGATTGTTGCC	−43	-1.30^{30}
DVU1164	Aliphatic amidase, *amiE*	TTGGCACACTTGTCGCT	−192	1.38^{90}
DVU1165	NADH respiratory dehydrogenase, *ndh*	CTGTCACGCCGGTTGCA	−109	1.32
DVU1258	Glutamine synthetase, type III, *glnN*	TTGGCCCGCTCTTTGCT	−26	1.13^{90}
DVU1259	Hypothetical protein	TTGGCCCGCTCTTTGCT	−99	2.09
DVU1441	Flagellin, *flaB1*	TTGGCATCGTGTTTGCT	−219	−1.32
DVU1442	Flagellin FlaG, putative	TTGGCATCGTGTTTGCT	−53	1.57
DVU1468	Peptidase/PDZ domain protein, *htrA*	CTGGCATGACCCCCGCT	−38	2.04
DVU1536	Transglycosylase, SLT family, *mltC*	CTGGCCCGGATGTTGCT	−36	-1.25^{30}
DVU1621	Hypothetical protein	TTGTCATGGCTGTTGCC	−85	−0.97
DVU1741	Hypothetical protein	CTGGCACTCTCCTTGCG	−70	1.22^{90}
DVU1805	GGDEF domain protein	ATGGCCCGCATATTGCT	−102	−1.16
DVU1831	Transporter, putative, authentic frameshift	TTGGGCCGCCGGTTGCA	−69	-1.16^{120}
DVU1881	PhoH family protein, *phoH*	TTGGCATGATTCATGCT	−67	−1.2
DVU2090	EF hand domain protein	AAGGTACACACCTTGCT	−10	1.08
DVU2106	Sigma 54-dependent transcriptional regulator, *flrC*	GTGGAACGGAACGTGCT	−59	-1.29^{60}
DVU2133	Membrane protein, putative	ATTGCACGCTTCTTGCT	−63	1.95
DVU2191	Hypothetical protein	GAGGCACACCCGTTGCT	−300	1.51^{60}
DVU2213	Nuclease domain protein	ATGCCACGCCCCCTGCA	−184	2.29^{60}
DVU2232	Hypothetical protein	ATGGAGCGTTTCTTGCT	−251	-0.97^{60}
DVU2359	Sigma 54-dependent transcriptional regulator	TTGGCACACCCCTTGCT	−183	3.25
DVU2471	Oxldoreductase, selenocysteine containing	AGGGCGCGGGCGTTGCA	−283	−1.55
DVU2489	Hypothetical protein	CTCGCACTGCTCTTGCG	−8	2.21

Continued on following page

TABLE 3—*Continued*

Promoter or site and DVU no.	Description	Site	Start	Log_2R
DVU2548	Acyl carrier protein phosphodiesterase, *acpD*	TTGGGACGGCGTATGCG	−11	3.65
DVU2652	Hypothetical protein	ATGGCACAGTGTATGCT	−47	1.59
DVU2653	Hypothetical protein	ATGGCACAGTGTATGCT	−99	1.80^{60}
DVU2659	ExsB protein	ATGGCCCGCATCTTCCA	−102	1.72
DVU2917	UDP-3-0-acyl *N*-acetylglucosamine deacetylase, *lpxC*	CTGGCACATCTTTTGCT	−169	−1.28
DVU2949	Membrane protein, putative	ACGGCATGGCGCTTGCG	−88	-1.40^{30}
DVU2951	Glutaminyl-tRNA synthetase, *glnS*	ATGGCAAGCACTTTGCG	−40	-1.36^{120}
DVU2956	Sigma 54-dependent transcriptional regulator, *flrA*	CTGGCACGGAACTTGTT	−64	2.34
DVU2957	Hypothetical protein	CTGGCACGGAACTTGTT	−46	−1.45
DVU2988	Phage shock protein A, *pspA*	CCGGCACGCTTCGTGCT	−121	0.90^{50}
DVU3020	Hypothetical protein	ATGGCATGTGCCTTGCT	−113	-1.40^{90}
DVU3120	Hypothetical protein	TTGGCACGCATCATGCT	−139	1.25^{90}
DVU3142	Sigma 54-dependent transcriptional regulator	CAGGCACAGTTCCTGCT	−66	1.05^{30}
DVU3143	Iron-sulfur cluster-binding protein	CAGGCACAGTTCCTGCT	−234	1.05^{120}
DVU3177	Hypothetical protein	CTGGCACGACTGCTGAA	−43	−1.39
DVU3202	Hydrolase, TalD family	GCGGCATGCGGTTTGCG	−73	3.58
DVU3234	Flagellar biosynthetic protein FliR	GTGGCCCGTATTTTGCT	−77	-1.69^{120}
DVU3235	IMP cyclohydrotase, putative *purH*	GTGGCCCGTATTTTGCT	−28	−1.97
DVU3283	Hypothetical protein	TTGGCACGGTTGGTGCT	−120	3.13
DVU3314	Peptidase, U32 family	GTGGCGTGGTGTTTGCG	−184	1.99
DVU3315	Dihydroorotate dehydrogenase, electron transfer subunit, *pyrK*	GTGGCGTGGTGTTTGCG	−37	1.92
DVU3384	Zinc resistance-associated protein, *zraP*	TTGGCACGCTCCATGCT	−38	1.48

[a] Expression ratios (log_2R) are shown for $Z \geq 1.5$ (absolute value) at 15 min unless otherwise indicated. For genes with $Z < 1.5$ (absolute value) at 15 min, data are shown for a different time point (appears as an italic superscript). σ^{54} promoter sequences were identified using PromScan (http://molbiol-tools.ca/promscan/). If the predicted site was located in the intergenic region between two divergent genes, both genes are included in the list. The CIRCE element upstream of *hrcA* (DVU0813) was identified using MEME (http://meme.nbcr.net/meme/website/intro.html) (3). Start sites are given as nucleotide numbers, with 0 being the first base of the predicted start codon of the protein. ND, data not available at any time point.

In contrast, heat shock genes in the gram-positive bacterium *B. subtilis* are assigned into six classes. The first three are the HrcA regulon (class I), the SigB regulon (class II), and the CtsR regulon (class III). Class V is regulated by a two-component signal transduction system, while class IV and class VI are controlled by unknown transcriptional activators (35). Whereas the *D. vulgaris* genome codes for an *hrcA* homolog, corresponding versions of *sigB* and *ctsR* seem to be lacking in this organism. The HrcA repressor protein binds to conserved *cis*-acting regulatory sequences, known as CIRCE elements, with the consensus sequence TTAGCACTC-N9-GAGTGCTAA commonly detected in several bacteria (45).

We analyzed the *D. vulgaris* genome sequence for the presence of heat shock promoter elements corresponding to the DNA binding sites for alternate sigma factors σ^{32} (RpoH) and σ^{54} (RpoN), apparently located in the genome (DVU1584 and DVU1628, respectively), as well as for the presence of CIRCE elements. Candidate binding sites for a variety of stress conditions in metal-reducing δ-proteobacteria were identified recently (31). For heat shock, four σ^{32} promoters and a single CIRCE element were reported (31). Here we report the presence of σ^{54} promoters identified using PromScan (http://molbiol-tools.ca/promscan/) with the default cutoff score of 80 and an additional CIRCE element (*P* value of 7.67e-08) identified upstream of *hrcA* (DVU0813) using MEME (http://meme.nbcr.net/meme/website/intro.html) (3). Results from both studies are summarized in Table 3.

σ^{32} promoters were identified upstream of DVU1003 (*dnaJ*), DVU1334 (trigger factor), the *rpoH* (DVU1584; σ^{70} family) operon, and the *groES groEL* operon (DVU1976, 60-kDa chaperonin; DVU1977, 10-kDa chaperonin) (31). Unlike *E. coli* and *B. subtilis*, the *D. vulgaris* genome apparently contains at least three orthologs of the gene for the molecular chaperone DnaJ, DVU1003 (COG2214), DVU1876 (COG484), and DVU3243 (COG484). DVU1876 belongs to the three-gene operon *dnaJ-clpB*, whereas DVU3243 belongs to the two-gene operon *rpoZ-dnaJ*. Both of these operons remained up-regulated during heat shock. Although we did not obtain reproducible signals for DVU1003 (Table 3), other genes in the same operon remained down-regulated throughout heat treatment. Also, reproducible signals were not obtained for *rpoH* (DVU1584); however, DVU1583, DVU1582, and DVU1581, predicted to be in the same operon, remained down-regulated. The three remaining genes in the predicted *rpoH* operon were surprisingly strongly increased in expression. The gene coding for the trigger factor, DVU1334, also remained down-regulated, in contrast to the increased expression of the genes for two ATP-dependent proteases, *clpX* and *lon*, downstream in the predicted four-gene operon. We did not obtain reproducible signals from microarray experiments for the *groES groEL* operon when expression ratios were calculated from treatment and control samples compared at the same time point. Expression ratios calculated from treatment samples at 60 min compared to control samples at 0 min revealed that *groES* ($log_2R = 3.69$; $Z = 4.59$) and *groEL* ($log_2R = 2.52$; $Z = 3.06$) were both up-regulated. Furthermore, proteomics analysis (discussed below) revealed that the protein levels of the 10-kDa chaperonin, GroEL (DVU1976), and the 60-kDa chaperonin, GroES (DVU1977), were enhanced at 60 min of heat stress. From Table 3, it appears that at least three of the four putative σ^{32}-promoted genes may be down-regulated during heat shock in *D. vulgaris*, suggesting either an alternate role for σ^{32} in

D. vulgaris or the presence of complex regulatory mechanisms in this organism.

The genome sequence of *D. vulgaris* reveals the presence of at least 37 σ^{54}-dependent transcriptional regulators (19), whereas the *E. coli* genome has 13 such genes (6, 38). Potential σ^{54} promoters have a well-conserved sequence that can be represented as YTGGCACGrNNNTTGCW (4). Upstream sequences of at least 98 genes from *D. vulgaris* were identified with putative σ^{54} promoter sites (Table S2 in the supplemental material). Table 3 lists 70 such genes with reproducible signals. Up-regulated genes in this list include those encoding σ^{54}-dependent transcriptional regulators (DVU2359 and DVU2956), peptidases (DVU0759, DVU1468, and DVU3314), flagellar motor switch protein (DVU0910), and the phage shock protein A (DVU2988) besides others. Although the physiological function of the phage shock operon is unclear, we note that σ^{54} has been reported to promote the transcription of the phage shock operon during heat shock in *E. coli* (44), similar to the result seen here.

Of the 70 genes with potential σ^{54} binding sites in the upstream region, 41 genes were increased in expression while 29 genes were down-regulated (Table 3). The products of σ^{54}-dependent regulation span a wide variety of functions, including utilization of nitrogen and carbon sources, energy metabolism, RNA modification, chemotaxis development, flagellation, electron transport, and response to heat and phage shock (8). σ^{54}-Controlled transcription proceeds only when the σ^{54}-RNA polymerase holoenzyme bound to the promoter site interacts with an activator through DNA looping (47) and thus allows for a large range of activator-dependent transcription activity at the same promoter (8, 42). This might explain why some genes promoted by σ^{54} seemed to be up-regulated while others were down-regulated. Analysis of the conserved σ^{54} promoter region predicted upstream of 98 genes from *D. vulgaris* revealed an unusual feature. Promoter sites for five σ^{54}-dependent transcriptional regulators and 36 other genes were predicted to be in reverse orientation relative to the promoted transcript, an unusual observation considering the unidirectional nature of transcriptional initiation (8). Analysis of the genome sequence of *D. alaskensis* G20 (closely related to *D. vulgaris*) similarly revealed the presence of at least five promoter sites predicted to be in reverse orientation relative to the promoted transcript (data not shown). No correlation was apparent between transcript levels and promoter orientation based on this data set (70 genes). Further studies will be needed to ascertain the role of reverse promoter orientation in σ^{54}-controlled transcription.

Candidate CIRCE sites for the negative regulator HrcA were found upstream of two operons, the *groES groEL* operon (31) and the *hrcA* operon (DVU0813). In *B. subtilis*, repressor activity of HrcA has been shown to be modulated by the GroE chaperonin system (35). During heat shock, misfolded proteins compete for the GroE chaperones that are necessary to fold nascent HrcA. Because of the rapid turnover of HrcA, the pool size decreases rapidly when GroE is unavailable, allowing transcription of HrcA-repressed genes. Interestingly, the *groES groEL* operon is also preceded by a σ^{32}-type promoter sequence. The predicted *hrcA* operon in *D. vulgaris* is apparently autogenously regulated and differs from that in *B. subtilis* in that it lacks *dnaJ*. It contains seven open reading frames and includes *hrcA*, *grpE* (DVU0812), and *dnaK* (DVU0811), all of which remained up-regulated during heat shock. We did not obtain reproducible signals for the remaining four genes in this operon.

Transcriptional response: comparison with *E. coli* and *B. subtilis*. The presence of promoter elements corresponding to σ^{54}, σ^{32}, and HrcA led us to probe the *D. vulgaris* genome for the presence of regulated heat shock proteins already discovered in *E. coli* (30) and *B. subtilis* (20). The *E. coli* heat shock regulon consists of approximately 34 core genes, encoding mainly chaperones and proteases, under the control of alternate sigma factors RpoH, RpoN, and RpoE. The *D. vulgaris* genome appears to carry at least 26 genes homologous to the *E. coli* heat shock regulon, the exceptions being *fkpA*, *hslO*, *htgA*, *htrC*, *metA*, *prpA*, and *rpoE* (σ^{24}). Table 4 compares early heat shock transcriptional response data from *D. vulgaris*, *E. coli*, (16) and *B. subtilis* (20) for genes homologous to the *E. coli* and *B. subtilis* heat shock regulons.

For most genes corresponding to the *E. coli* heat shock regulon, the transcriptional response of *D. vulgaris* seemed to agree well with the other organisms, the notable exception being DVU2260 (*rrmJ*), the gene encoding the 23S rRNA methylase (COG293). Note that COG293 as a gene family is absent in the *B. subtilis* genome. *rrmJ* is the first gene of a three-gene operon also containing a gene that encodes a conserved hypothetical protein (DVU2259) and *ruvC* (DVU2258; crossover junction endodeoxyribonuclease), all of which remained down-regulated at near constant levels throughout heat exposure. The corresponding *rrmJ* in *E. coli* (b3179) remained up-regulated and occurs in the same operon as *hflB* (alternatively named *ftsH*), encoding the ATP-dependent Zn protease. The latter gene remained up-regulated in *E. coli* and *D. vulgaris*. Methylation of 23S rRNA affected by RrmJ has been suggested to alter translation rates, modulate rRNA maturation, or affect the stability of rRNA structures (9). Although the mechanism of stress-dependent activity of RrmJ is unclear (9), methylation of rRNA during heat shock might play a different role in *D. vulgaris* than in *E. coli*.

Of the six classes of heat shock genes in *B. subtilis*, two (classes I and III) are controlled by transcriptional repressors, one (class II) by an alternate sigma factor, and one (class V) by a two-component signal transduction system. Class IV and class VI are controlled by unknown transcriptional activators (35). Class I genes belong to the *hrcA* operon, the repressor for which, HrcA, is absent in *E. coli*. Expression patterns of most genes in this class agreed well between *D. vulgaris* and *B. subtilis*, the sole exception being DVU2339 (*prmA*, ribosomal protein L11 methyltransferase; data not shown), which remained down-regulated. Whereas *yqeT*, the ortholog in *B. subtilis*, occurs in the *hrcA* operon, DVU2339 occurs in an entirely different three-gene operon, all of which remained down-regulated. The *hrcA* operon in *D. vulgaris* also lacked *dnaJ*. However, two copies of *dnaJ* (DVU1876 and DVU3243) did occur in *D. vulgaris* in different locations and remained up-regulated.

Class II genes from *B. subtilis* represent the largest group of stress response genes that respond to heat shock in this organism and offer protection under a variety of other stress conditions, such as salt, oxidation, acid stress, and starvation for glucose (35). Transcriptional responses for several genes in this class agreed well with the corresponding homologs from *D. vulgaris*, the exceptions being DVU1586 (thiol-disulfide isomerase), DVUA0091 (catalase), DVU1449 (anti-anti-sigma fac-

TABLE 4. Comparison of transcriptional response ratios (log_2R) during early heat shock conditions for *D. vulgaris* (15 min, 13° upshift), *B. subtilis* (3 min, 11° upshift) (20), and *E. coli* (5 min, 13° upshift) (16)[a]

COG	Function	*D. vulgaris* Hildenborough homolog	Log_2R	*B. subtilis* MO945 homolog	Log_2R	*E. coli* K12 homolog	Log_2R	Comment(s)
COG71	Molecular chaperone (small heat shock protein), Hsp20 family	DVU2442	3.66	Bsu1798	1.59	b3686 b3687	5.13 5.1	*E. coli* σ^{32} regulon
COG293	23S rRNA methylase, *rrmJ*	DVU2260	−1.65	NH	NH	b3179	1.92	
COG465	ATP-dependent Zn proteases, *ftsH*	DVU1278	2.55	Bsu1243	−1.48	b3178	1.13	
COG443	Molecular chaperone, *dnaK*	DVU0811	2.94	Bsu2543	1.48	b0014	2.35	*E. coli* σ^{32} regulon, *B. subtilis* class I
COG484	DnaJ-class molecular chaperone with C-terminal Zn finger domain, *dnaJ*	DVU1876 DVU3243	0.83 1.57	Bsu2542	0.83	b0015	2.91	
COG576	Molecular chaperone GrpE (heat shock protein), *grpE*	DVU0812	1.83	Bsu2544	1.35	b2614	2.04	
COG1420	Transcriptional regulator of heat shock gene, *hrcA*	DVU0813	2.68	Bsu2545	2.23	NH	NH	*B. subtilis* class I
COG463	Glycosyltransferases involved in cell wall biogenesis	DVU3013 DVU1892	−2.08 −0.96	Bsu1975 Bsu3552 Bsu0859	−0.99 1.17 2.79	b2254 b2351	−2.65 −1.89	*B. subtilis* class II
COG28	Thiamine pyrophosphate-requiring enzymes, *lvlB*	DVU0360	1.27	Bsu2827 Bsu3599	−1.98 3.7	b3671	1.85	
COG693	Putative intracellular protease/amidase, Pfpl family	DVU1933	−1.22	Bsu2698	−1.53	b1967	2.46	
COG1366	Anti-anti-sigma regulatory factor	DVU1449	−1.23	Bsu2474	2.84	NH	NH	
COG2814	Arabinose efflux permease	DVUA0096	2.62	Bsu3393 Bsu0740 Bsu3754 Bsu3506 Bsu3143 Bsu1854 Bsu0854 Bsu4081 Bsu3977 Bsu3581	0.82 0.98 1.17 1.16 1.17 1.54 1.56 1.63 3.93 4.05	b1981 b1528 b2587	−1.83 −2.04 −2.3	
COG542	ATPases with chaperone activity, ATP-binding subunit, *clpA/B*	DVU1602 DVU1874	2.8 4.24	Bsu0086	3.04	b0882	2.73	*E. coli* σ^{32} regulon, *B. subtilis* class III

[a] Genes were chosen for comparisons for which data were available in all three organisms. Data shown are for *D. vulgaris* only for $Z \geq 1.5$ (absolute value). ND, data not available. NH, no homolog in that organism corresponding to the specific COG.

tor), and DVU1574 (ribosomal protein L25). Even though several orthologs of class II genes from *B. subtilis* were up-regulated, we could not identify any *sigB*-type promoter sequences upstream of these genes in *D. vulgaris*. In *B. subtilis*, *sigB* occurs in an octacistronic operon in a penultimate promoter-distal position, with *rsbX* being promoter distal and the six genes *rsbR* through *rsbW* being promoter proximal. Interaction of SigB with the RNA polymerase core is controlled by RsbW (anti-sigma factor) and RsbV (anti-anti-sigma factor) under stressed and nonstressed conditions. Orthologs to *rsbW* (DVU1450), *rsbV* (DVU1449), and *rsbU* (DVU1451) are apparently in the *D. vulgaris* genome in the vicinity of each other, but an ortholog to *sigB* cannot be identified and may be absent. The anti-anti-sigma factor (DVU1449) and anti-sigma factor (DVU1450) were down-regulated during heat shock.

Class III genes contain eight genes from the *ctsR* regulon of *B. subtilis*. This includes *clp* ATP-dependent proteases, *clpP* and *clpE* (two monocistronic operons), in addition to the hexacistronic *ctsR* operon, which carries a gene that encodes another ATP-dependent protease, *clpP* (20, 35). Available data for two orthologs from this class, DVU1602 and DVU1874, agreed well with the corresponding genes from all four organisms. Class IV is comprised of just one gene, *htpG*, whereas class V has two members, *htrA* and *htrB* (*yvtA*) (10). The *D. vulgaris* genome has two versions of *htrA* (DVU1468 and DVU3278), both belonging to COG265. Reproducible signals were obtained for DVU1468 and agreed well with expression data for serine proteases from *B. subtilis*.

From the comparative analyses of the three organisms, it is apparent that the *D. vulgaris* genome has several conserved heat shock genes and shares regulatory features with those of *E. coli* and *B. subtilis*. Also, while mechanisms of heat shock control for a few genes appear to coincide with those established for *E. coli* and *B. subtilis*, *D. vulgaris* also possesses unique control schemes for several other genes that respond to this stress.

Comparison of transcriptional and translational responses. The primary mode of regulation of gene expression in bacterial systems is through transcription. Global proteomics tools serve to complement transcriptional analyses as well as to provide clues on translational control mechanisms, if they exist. We used DIGE analyses of the soluble proteome from *D. vulgaris*

TABLE 5. Comparison of transcript abundance with corresponding protein levels following heat shock of *D. vulgaris*[a]

DVU no.	Description	Expression ratio	
		Protein	mRNA
DVU0160	Carbohydrate isomerase, KpsF/GutQ family	4.16	2.33
DVU0811	DnaK protein, *dnaK*	6.57	1.63
DVU0847	Adenylyl-sulphate reductase, alpha subunit, *apsA*	−2.92	−1.79
DVU0910	Flagellar motor switch protein, *fliM*	3.08	3.15
DVU1468	Peptidase/PDZ domain protein, *htrA*	3.92	1.60
DVU1636	Inorganic pyrophosphatase, manganese dependent, *ppaC*	−4.62	−1.69
DVU1772	Pyridine nucleotide-disulfide oxidoreductase, *gltD*	−4.41	−1.18
DVU2247	Alkyl hydroperoxide reductase C, *ahpC*	6.64	1.83
DVU2548	Acyl carrier protein phosphodiesterase, *acpD*	4.28	2.63
DVU2643	Heat shock protein, *htpG*	2.75	1.33
DVU2744	High-affinity branched-chain amino acid ABC transporter	5.00	2.55
DVU0095	Polyamine ABC transporter, periplasmic polyamine-binding protein, *potD-1*	5.94	−1.62
DVU0177	Molybdenum ABC transporter, periplasmic molybdenum-binding protein, *modA*	3.38	−2.33
DVU0712	Amino acid ABC transporter, periplasmic-binding protein	2.73	−1.31
DVU0745	ABC transporter, periplasmic substrate-binding protein	3.97	−2.24
DVU0966	Amino acid ABC transporter, periplasmic amino acid-binding protein	2.88	−2.70
DVU1932	Adenylate kinase, *adk*	2.62	−3.87
DVU2667	Phosphate ABC transporter, periplasmic phosphate-binding protein, *pstS*	3.53	−1.72
DVU3245	Transcription elongation factor, *greA*	−3.49	2.11
DVU0322	Enolase, *eno*	3.20	ND
DVU0386	Amino acid ABC transporter, periplasmic amino acid-binding protein, *glnH*	3.87	ND
DVU0415	Cytosol aminopeptidase, *pepA*	18.05	ND
DVU0750	Methyl-accepting chemotaxis protein	3.87	ND
DVU0851	Hypothetical protein	−2.78	ND
DVU0978	ABC transporter, periplasmic substrate-binding protein	3.20	ND
DVU1976	Chaperonin, 60 kDa, *groEL*	4.90	ND
DVU1977	Chaperonin, 10 kDa, *groES*	4.03	ND
DVU2138	Conserved hypothetical protein	2.85	ND
DVU2427	Hypothetical protein	2.52	ND
DVU2649	Hypothetical protein	2.65	ND
DVU3061	Sensory box histidine kinase	4.40	ND
DVU3150	Ribosomal protein S1, *rpsA*	3.00	ND

[a] Heat-shocked cells (60 min, 50°C) and control samples were extracted for mRNA and soluble proteins. Protein levels were determined from Ettan DIGE (Amersham) of total soluble protein, while transcript abundance was determined from cDNA microarray analysis. Selected proteins were identified by MASCOT search analysis with a MOWSE score of ≥60 (27). Transcript abundance ratios were reported only for ratios with a significance of $Z \geq 1.5$ (absolute value). ND, data not available. Expression ratios for proteins are fold ratios, and those for mRNA transcripts are $\log_2$ ratios.

to compare protein levels to mRNA levels between cells subjected to heat stress for 60 min and comparable control cells. A total of 239 spots that displayed expression ratio differences of twofold or higher from three 2-D gels were selected for identification by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) analysis. MS analyses resulted in identification of 32 proteins with MOWSE scores of 60 or higher (Table 5). Log_2R values for the corresponding transcripts are also presented. Proteins overexpressed twofold or greater under heat shock (27) seemed to be overrepresented compared to those repressed twofold or more (5). Note that the number of up-regulated genes (248) at 60 min was also higher than that of down-regulated genes (145) (Fig. 1). Proteins listed in Table 5 are arranged into three groups based on comparison with transcript ratios. The first group lists proteins for which there was good correlation between the protein expression ratios and $\log_2$R values for the corresponding transcripts. Overexpressed proteins included common chaperones and proteases, DnaK (DVU0811), HtpG (DVU2643), HtrA (DVU1468), AhpC (DVU2247), and others, whereas ApsA (DVU0847), PpaC (DVU1636), and GltD (DVU1772) were underexpressed. For the third group of proteins, mRNA transcript ratios were not available. Notable in this list were the chaperonins GroES (DVU1977) and GroEL (DVU1976). The chaperones DnaK (DVU0811), GroES (DVU1977), and GroEL (DVU1976) and the antioxidant AhpC (DVU2247) appeared as multiple spots with slightly different molecular weights and pIs. 2-D gel analysis of heat shock stress in *Agrobacterium tumefaciens* also revealed the presence of additional forms of DnaK which were absent during balanced growth (32). The presence of multiple spots or opposite trends in the protein and mRNA levels might suggest heat shock-dependent posttranslational modifications (33). Common posttranslational modifications known to be involved in heat shock-related processes include phosphorylation, protein oxidation, sulfoxidation, and acetylation (33, 36). MALDI-TOF MS analysis has the capacity to identify the nature of the modification and the modified peptide but not the exact amino acid. When the MALDI-TOF MS spectra for the proteins exhibiting aberrant migration patterns were analyzed, evidence for posttranslational modifications was not found.

The last group of proteins in Table 5 displayed expression ratios that were not in agreement with the mRNA ratios. Most proteins in this list were ABC transporters, including those for amino acids (DVU0712 and DVU0966), polyamines (DVU0095), molybdenum (DVU0177), and phosphate (DVU2667) displaying increased expression ratios in contrast to the transcript values. The overabundance of

periplasmic ABC transporters displaying enhanced expression after heat shock might be attributed to an experimental artifact resulting from enhanced extraction of these proteins from stressed cells. Further studies will be needed to explore this possibility.

Summary. Results presented in this work represent the first example of global heat shock in *D. vulgaris*. Temporal gene expression profiles suggest the presence of both direct and indirect sensing mechanisms for coping with temperature upshift. The indirect sensing mechanism may, at least in part, be attributed to the presence of *hrcA*, encoding a putative transcriptional regulator of heat shock genes. The role of the alternate sigma factor σ^{32} seems unclear in this organism, because several genes apparently regulated by this factor have lowered expression in response to a temperature increase. The other alternate sigma factor, σ^{54}, likely controls the expression of a large number of genes during heat shock, possibly through complex regulatory mechanisms. While *D. vulgaris* apparently shares certain regulatory features with *E. coli* and *B. subtilis* because expression of several COGs conserved between the three organisms seemed coherent, it must also possess unique mechanisms for controlling transcription of a large number of genes. Comparison of proteins expressed during heat shock confirmed roles of several HSPs and suggested possible post-translational modifications for a few. Work is currently under way to elucidate unique mechanisms of heat shock control in this SRB.

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