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NOTES

Dynamic Metabolic Adjustments and Genome Plasticity Are Implicated in the Heat Shock Response of the Extremely Thermoacidophilic Archaeon *Sulfolobus solfataricus*†

Sabrina Tachdjian and Robert M. Kelly*

Department of Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, North Carolina 27695-7905

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Approximately one-third of the open reading frames encoded in the *Sulfolobus solfataricus* genome were differentially expressed within 5 min following an 80 to 90°C temperature shift at pH 4.0. This included many toxin-antitoxin loci and insertion elements, implicating a connection between genome plasticity and metabolic regulation in the early stages of stress response.

The ability to cope with environmental stress is essential to microorganisms (1, 34, 39), including those thriving in extreme environments relative to temperature (4, 10, 21, 28, 42, 45, 50, 52), pressure (9, 31, 35, 44, 51), ionic strength (47), acidity (30, 53), alkalinity (26), metals (15, 37), and radiation (25, 36, 43). Certain crenarchaea, such as members of the *Sulfolobales*, occupy niches that are biologically extreme in two respects: low pH and elevated temperature (19). Key to their physiological function is a transmembrane proton gradient that renders intracellular pH close to neutral. As such, maintaining cytosolic pH in the face of thermal stress-induced cellular damage involves complex genetic and metabolic strategies (40). To examine such mechanisms for extreme thermoacidophiles at supraoptimal temperatures, the heat shock response of *Sulfolobus solfataricus* (49, 55) was studied using genome-wide transcriptional response.

A whole-genome oligonucleotide microarray for *Sulfolobus solfataricus* P2 (DSMZ, Germany) was developed (49). Probes were designed in OligoArray 2.0 (46), custom synthesized (Integrated DNA Technologies, Coralville, IA), and printed onto arrays following protocols previously developed for other hyperthermophiles (14, 20, 50); five replicates per probe were spotted on each array to fortify statistical analysis. *S. solfataricus* was routinely grown at 80°C and pH 4.0 on DSMZ 182 medium; cells were enumerated using epifluorescence microscopy with acridine orange stain (13). The heat shock time course experiment was carried out as described in the legend for Fig. 1A. RNA was extracted from chilled culture samples (12). cDNA synthesis, microarray hybridizations (Fig. 1B), and data collection were performed as described previously (12), with minor adjustments for long oligonucleotide platforms. Data from each experiment were analyzed with SAS 9.0 (SAS, Cary, NC) (42), using a mixed linear analysis of variance model (54). A ±2.0-fold change (FC) or higher defined differential expression.

Transcriptional response to heat stress. When *S. solfataricus* was shifted from 80 to 90°C at pH 4.0, approximately one-third of the genome responded (1,088 genes, 551 up/537 down) within 5 min after the culture reached 90°C. Differential expression was less pronounced after this initial period; ~300 genes (161 up/144 down) changed between 5 and 30 min, and only 30 genes (18 up/12 down) changed between 30 and 60 min (Table 1 and Fig. 2). Table 2 lists selected heat shock (HS)-responsive genes involved in basic metabolic functions and regulation. *S. solfataricus* relies on HSP20 family small heat shock proteins (sHSPs) (27), the thermosome/rosettasome (21) for protein folding, and the proteasome (33), several HtpX homologs (48), and various other proteases for protein turnover. Here, both sHSPs responded within 5 min after temperature shift (Table 3). In contrast, the α and β thermosome subunits were not HS responsive; this was expected given their already high expression levels under normal conditions (23). The γ thermosome subunit expression, however, was significantly lower than those for the α and β subunits before stress and was further down-regulated during the course of HS response. This is consistent with previous reports showing a shift in thermosome composition from 1α:1β:1γ to a heat-stressed ratio of 2α:1β:0γ (22). Genes encoding HtpX proteases and proteasome subunits (α, β1, and β2) were not affected by HS, and the proteasome-associated nucleotidase was down-regulated significantly. Genes encoding subunits of the exosome, which is involved in mRNA polyadenylation and degradation

* Corresponding author. Mailing address: Dept. of Chemical and Biomolecular Engineering, North Carolina State University, EB-1, 911 Partners Way, Box 7905, Raleigh, NC 27695-7905. Phone: (919) 515-6396. Fax: (919) 515-3465. E-mail: rmkelly@eos.ncsu.edu.

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

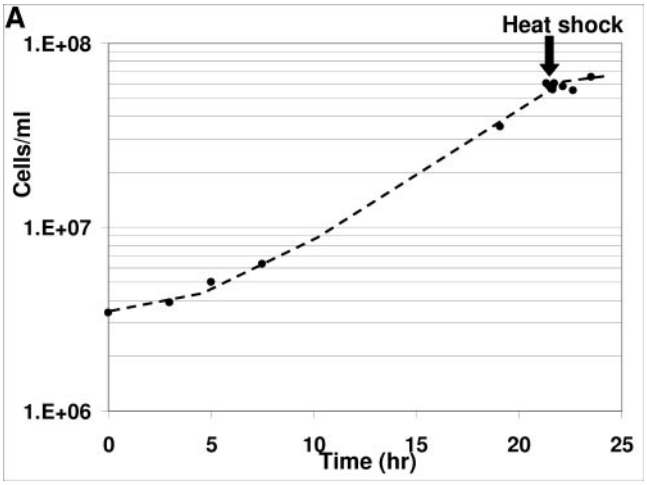

FIG. 1. Cell density of *S. solfataricus* before and during HS (A) and experimental design used for microarray hybridizations (B). The experiment was carried out with a modified 3-liter glass fermentor (Applikon, Schiedam, The Netherlands). The culture was shifted from 80 to 90°C (~8 min) at mid-exponential phase and maintained at 90°C ± 1°C for 2 h. Samples were taken 10 min before starting the temperature shift and then 5, 30, and 60 min after reaching 90°C. cDNA samples were then hybridized in a four-slide loop design (B). Dots and arrowheads represent cyanine 3- and cyanine 5-labeled samples, respectively.

(41), were strongly repressed immediately after HS (Table 3). Three open reading frames (ORFs) encoding Sso7d DNA binding proteins were up-regulated upon heat shock, consistent with their role in maintaining negative supercoiling of DNA during thermal stress (29). Many transcriptional regulators were strongly induced by thermal stress (Table 2), consistent with widespread changes in the *S. solfataricus* transcriptome. The most significant changes were for putative TetR (SSO2506, +24.3 FC) and GntR family repressors (SSO1589, +32.0 FC); strong induction of both within 5 min indicates an important role in early HS response.

IS elements, transposes, and resolvases. The *S. solfataricus* genome encodes more than 200 insertion sequence (IS) elements and associated fragments, which, taken together, represent approximately 10% of the genome (5). IS elements and miniature inverted-repeat transposable elements (MITEs) are thought to be responsible for genome shuffling in *S. solfataricus* and *S. tokodai*. In sharp contrast, *S. acidocaldarius* contains no IS elements (6). Since the presence of multiple (almost identical) copies of IS element-related sequences complicated gene expression analysis in some cases, the entire subset of these ORFs was treated as a group. Extensive differential expression of IS sequences, transposases, and resolvases (a group of ~400 ORFs) was noted under HS, with ~60% of these ORFs differentially expressed for at least one time point comparison. This proportion was more than twice that observed during unstressed growth (unpublished data), implicating HS in IS element differential expression. These ORFs typically responded early, with as many as 208 ORFs (203 up/5 down) differentially expressed within 5 min after HS, representing no less than ~37% of all up-regulated genes for this time point comparison and indicating a crucial role for transposition and genome plasticity (24) in early HS response. The mobility of IS elements can increase genetic diversity in the face of stress (16) by either causing mutations (11, 32), moving genes to a different chromosomal location, deleting or inverting ORFs, inactivating some by insertion, or activating genes by positioning a promoter upstream of the coding region (3). Moreover, transposon mutagenesis was

TABLE 1. Summary of differentially transcribed ORFs in *S. solfataricus* under heat shock

ORFs	No. of responsive genes by time point comparison			
	5 vs −10 min	30 vs 5 min	60 vs 30 min	60 vs −10 min
Up-regulated	551[a]	161	18	489
Down-regulated	537	144	12	391
Total	1,088	305	30	880

[a] Of which 205 genes are IS elements, transposases, resolvases, and related sequences.

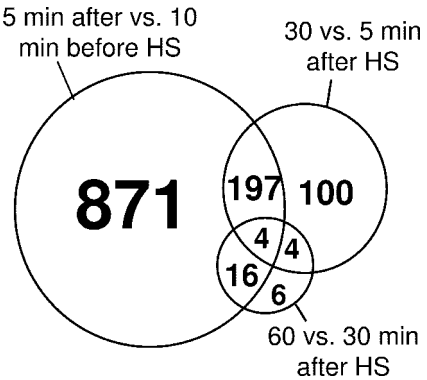

FIG. 2. Venn diagram representing the overlap of differential gene expression between time point comparisons for heat shock-responsive genes. Note that the numbers in each circle are additive.

TABLE 2. Characteristics of selected heat shock-responsive genes for *S. solfataricus* cultured at pH 4.0 and 80°C and shifted to 90°C

Metabolic pathway/function	ORF, gene name or description	Change (*n*-fold)	Time point comparison (min)
DNA metabolism			
DNA polymerase II	SSO1459	−4.0	60 vs −10
	SSO8124	−12.1	60 vs −10
Recombinases (putative)	SSO2452 *recA*	+3.7	60 vs −10
	SSO1994	+3.5	5 vs −10
Double-stranded DNA break and recombination	SSO0264 putative *recB*	+2.0	60 vs −10
	SSO0250 *radA*	+2.3	60 vs −10
Partial exonuclease	SSO2250 *rad32*	+2.8	30 vs 5
	SSO2373	+2.1	60 vs −10
Damage-inducible DNA repair polymerase	SSO2448 *dinP*	+3.5	60 vs −10
Putative DNA ligase	SSO2734	+5.3	5 vs −10
Putative endonuclease	SSO3029	+2.8	60 vs −10
MutT-like protein	SSO3167	+3.2	60 vs −10
Transcription and DNA modification	SSO0265	+4.3	60 vs −10
	SSO0266, *tfe*	+5.6	60 vs −10
	SSO0267	+4.0	60 vs −10
	SSO0269, *hflX*	+2.3	60 vs −10
	SS0270	+3.0	60 vs −10
mRNA production			
RNA polymerase	SSO0223 *rpoA2*	−5.8/+3.4	5 vs −10/30 vs 5
	SSO0225 *rpoA1*	−2.2/+1.4	5 vs −10/30 vs 5
	SSO0227 *rpoB1*	−7.1/+2.5	5 vs −10/30 vs 5
	SSO3254 *rpoB2*	−4.8/+3.5	5 vs −10/30 vs 5
	SSO0071 *rpoD*	−12.6/+2.9	5 vs −10/30 vs 5
Energy metabolism			
NADH dehydrogenase	SSO0323 *nuoC*	−2.1/+3.0	5 vs −10/30 vs 5
	SSO0324 *nuoD*	−9.2/+5.6	5 vs −10/30 vs 5
	SSO0325 *nuoH*	−11.3/+4.0	5 vs −10/30 vs 5
	SSO0326, *nuoI*	−10.6/+5.3	5 vs −10/30 vs 5
	SSO0327, *nuoJ*	−6.7/+2.8	5 vs −10/30 vs 5
	SSO0328, *nuoL*	−6.5/+2.0	5 vs −10/30 vs 5
	SSO0329, *nuoN*	−7.0/+1.9	5 vs −10/30 vs 5
Flavoproteins	SSO0584	+5.7	5 vs −10
	SSO2348	−2.3	5 vs −10
	SSO2353	−3.2	5 vs −10
	SSO2762	+2.0	5 vs −10
	SSO2763	+2.3	5 vs −10
	SSO2776	+2.0	60 vs −10
	SSO2817	−5.3	5 vs −10
	SSO2819	−2.8	5 vs −10
Terminal oxidase	SSO0044 *doxB*	−4.9	5 vs −10
	SSO0045 *doxC*	−4.3	5 vs −10
	SSO5098 *doxE*	−2.5	5 vs −10
Miscellaneous redox proteins	SSO0368 *trxA-1*	+2.6	60 vs −10
	SSO2232 *trxA-2*	+2.6	60 vs −10
	SSO2416	+2.3	60 vs −10
	SSO2765	+3.2	60 vs −10
Cofactor biosynthesis			
Cobalamin	SSO2305, *cbiD*	−5.3	5 vs −10
	SSO2296 *cbiE*	−2.1	5 vs −10
	SSO2299 *cbiF*	−3.7	5 vs −10
	SSO2297 *cbiG*-like	−3.2	5 vs −10
	SSO2306 *cbiH*	−3.2	5 vs −10
	SSO2301 *cbiL*	+3.7	5 vs −10
	SSO2303 *cbiT*	+3.7	5 vs −10
MGD[a]	SSO0770 *moaC*	+2.0	30 vs 5
	SSO0675 *mobB*	+3.7	5 vs −10
	SSO0676	+2.0	60 vs −10
	SSO2365 *moeA*	+2.1	30 vs 5
Lipids			
Acetyl coenzyme A C-acetyltransferase	SSO0534 *acaB*-1	−6.5	60 vs −10
	SSO2062 *acaB*-3	−2.5	5 vs −10
	SSO2697 *acaB*-8	−3.2	60 vs −10
	SSO2377 *aca*-4	+3.7	60 vs −10
	SSO2496 *acaB*-5	+3.5	60 vs −10
	SSO2508 *acaB*-6	+2.0	60 vs −10
	SSO3113 *acaB*-10	−4.3	60 vs −10

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TABLE 2—*Continued*

Metabolic pathway/function	ORF, gene name or description	Change (*n*-fold)	Time point comparison (min)
Fatty acid biosynthesis	SSO1030 *fabG*-2	−3.0	60 vs −10
	SSO *fabG*-3	+4.3	5 vs −10
	SSO2205 *fabG*-4	−2.3	60 vs −10
	SSO2276 *fabG*-5	−2.3	5 vs −10
	SSO2500 *fabG*-7	+2.8	5 vs −10
	SSO3004 *fabG*-9	+3.5	5 vs −10
	SSO 3114 *fabG*-10	−3.0	60 vs −10
Transporters			
Iron	SSO0485	−4.3	60 vs −10
	SSO0486	−4.6	60 vs −10
	SSO0487	−4.0	60 vs −10
Cobalt	SSO1892	−4.9	60 vs −10
	SSO1893	−4.9	60 vs −10
	SSO1894	4.6	60 vs −10
Phosphate	SSO0489	−2.1	60 vs −10
	SSO0490	−2.5	60 vs −10
Sulfate	SSO2469	−4.0	5 vs −10
Amino acids	SSO0786	−2.3	60 vs −10
	SSO1009	−2.1	5 vs −10
	SSO1069	−6.5	5 vs −10
	SSO1173	−3.5	5 vs −10
	SSO2726	−3.0	5 vs −10
	SSO2728	−2.5	5 vs −10
Peptides	SSO3047	−2.0	5 vs −10
	SSO3048	−2.1	5 vs −10
Oligonucleotides/dipeptides	SSO1274	−16.0	5 vs −10
	SSO1275	−14.9	5 vs −10
	SSO1276 *oppD*	−7.5	5 vs −10
	SSO1277 *oppF*	−8	5 vs −10
	SSO1281	−2.5	5 vs −10
	SSO1282 *oppD*	−5.3	5 vs −10
	SSO1283	−6.5	5 vs −10
Arabinose	SSO3066	−5.6	60 vs −10
	SSO3067	−3.5	60 vs −10
	SSO3069	−2.0	60 vs −10
Glucose	SSO2848	−3.7	5 vs −10
	SSO2849	−4.3	5 vs −10
	SSO2850	−7.0	5 vs −10
Maltose	SSO1000	−3.5	5 vs −10
	SSO1170	−3.5	5 vs −10
	SSO3053	−2.5	60 vs −10
Drug resistance/efflux	SSO2035	+2.0	60 vs −10
	SSO2135	+4.6	60 vs −10
	SSO2137	+3.0	60 vs −10
	SSO2228	+5.3	5 vs −10
	SSO2704	+2.8	60 vs −10
	SSO3012	+4.3	60 vs −10
Transcriptional regulators			
TetR family repressor	SSO2506	+24.3	5 vs −10
GntR family repressors	SSO1255	+4.0	5 vs −10
	SSO1589	+32.0	5 vs −10
MarR/Lrs14 family repressors	SSO0048	+3.2	5 vs −10
	SSO0458	+4.0	5 vs −10
	SSO1082	+4.9	5 vs −10
	SSO1108	+4.0	5 vs −10
	SSO1110	+3.7	5 vs −10
	SSO2138	+3.0	5 vs −10
	SSO2897	+6.0	5 vs −10
	SSO3242	−10.6	60 vs −10
Miscellaneous regulators	SSO0200	−2.6	60 vs −10
	SSO0239	−3.2	5 vs −10
	SSO0618	+9.1	60 vs −10
	SSO0669	+2.1	60 vs −10
	SSO1952	−3.5	60 vs −10
	SSO2131	+2.5	5 vs −10
	SSO5522	+3.2	5 vs −10
	SSO10340	+2.8	5 vs −10
	SSO2827	+4.0	5 vs −10

[a] MGD, molybdopterin guanine dinucleotide.

TABLE 3. Characteristics of HS response of stress-related proteins in *S. solfataricus*[a]

Category	ORF, gene name (gene symbol)	Change (*n*-fold)	Time point comparison (min)
Chaperones, HSPs and chromatin proteins			
sHSP	SSO2427	+2.0	5 vs −10
	SSO2603	+3.2	5 vs −10
HtpX homolog	SSO1859	NC	
	SSO2694	NC	
	SSO3231	NC	
USP family	SSO0529	−2.0	5 vs −10
	SSO1865	+3.7	5 vs −10
	SSO2778	+3.0	5 vs −10
	SSO3183	+2.0	5 vs −10
Thermosome	SSO0862 (α)	NC	
	SSO0282 (β)	NC	
	SSO3000 (γ)	−3.0	60 vs −10
Sso7d	SSO9180	+4.0	5 vs −10
	SSO9535	+4.6	5 vs −10
	SSO10610	+4.6	5 vs −10
Protein and mRNA turnover			
Proteasome	SSO0738 (α)	NC	
	SSO0278 (β1)	NC	
	SSO0766 (β2)	NC	
	SSO0271 (PAN)	−7.2	5 vs −10
Exosome	SSO0732 *rrp42*	−6.5	5 vs −10
	SSO0735 *rrp41*	−4.3	5 vs −10
	SSO0736 *rrp4*	−10.6	5 vs −10

[a] NC, no change; PAN, proteasome-associated nucleotidase; USP, universal stress protein.

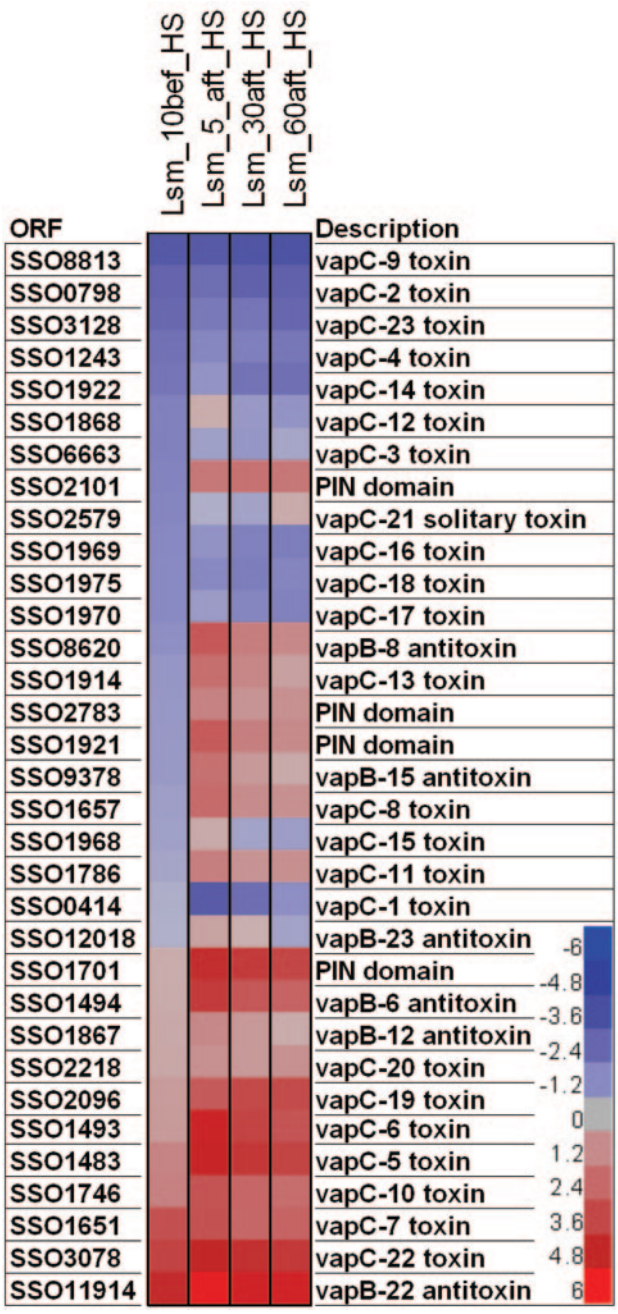

FIG. 3. Heat plot representing relative expression levels for toxins, antitoxins, and PIN domain-containing ORFs. Each column represents data for a given time point (from left to right, 10 min before HS and then 5, 30, and 60 min after reaching 90°C). Missing antitoxins correspond to ORFs that had not been reported in the initial genome sequence (49).Values are sorted according to the preshock time point. Data are centered on zero (gray), which represents the average gene expression level over the entire genome, where each unit above (red) or below (blue) equals one standard deviation from the average.

shown to be the cause of the high mutation rate observed in *S. solfataricus* (32). The fact that a selective agent, in this case HS, could directly increase genetic mutation and rearrangement rates in *S. solfataricus* suggests that mutations are not random and spontaneous (8).

Toxin-antitoxin loci also responded to HS. Only one toxin-antitoxin (TA) family (*vapBC*) has been identified in the *S. solfataricus* genome, represented by 22 TA pairs and 1 solitary toxin (38). Although first thought to be exclusively associated with postsegregational killing, recent studies have shown that TA loci are also involved in stress response (7) and trigger a reversible bacteriostatic effect rather than a lethal one (18). Toxins typically contain a PIN domain, which has been shown to function as an exonuclease (2). By cleaving mRNAs, toxins interfere with transcription, thereby modulating metabolic activity (2). Here, specific TA loci responded to HS, presumably to slow growth and thus minimize the burden of housekeeping functions. It has been proposed that TA loci are constitutively repressed (17), but here it was noted that, even under unstressed conditions, *vapBC* ORF expression levels differ, ranging from very low (*vapC*-9) to very high (*vapBC*-22) (Fig. 3). Consequently, not only was *vapBC* locus expression heat stress induced in *S. solfataricus*, but many loci were transcribed even under normal growth conditions. This suggests that (i) *vapBC* loci may play various roles in *S. solfataricus*, (ii) activation mechanisms (protease based) and toxin potency may differ from one TA pair to another, and (iii) cells may use TA systems to modulate their metabolic activity even in the absence of perturbations. Efforts are now under way to test these hypotheses.

While thermal stress response in *S. solfataricus* needs to be examined further, dynamic genome-wide differential expression analysis such as that reported here can lead to new insights

into the adaptive and governing mechanisms underlying extreme thermoacidophily.

Supplemental material. Comprehensive listings and data analysis for the dynamic heat shock experiments with *S. solfataricus* are reported as supplemental material.

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