SUPPLEMENTAL MATERIAL

Supplemental Results

hslR and *hslO* form a σ^{32}-dependent operon

Our RT-PCR experiments demonstrated that expression of heat shock genes is induced upon both heat stress and disulfide stress. However, induction by heat shock and by disulfide stress showed a strikingly different kinetic pattern. We therefore looked in more detail at the expression of *hslR* and *hslO* using Northern blots. A Northern blot with specific probes for *hslR* and *hslO* confirmed the increase of heat shock gene transcripts over time under disulfide stress. A ~1500 b transcript was detected with both probes in all conditions tested matching to the known σ^{32}-dependent transcript covering both genes (Fig. S1A) (1). In addition, we detected an individual *hslO* mRNA of ~900 b length with the *hslO* probe. This RNA could be generated from a predicted σ^{28} promoter (RegulonDB v. 8.1(2)) or represent a potential posttranscriptional cleavage product of the original transcript. The other part of this cleavage product would then correspond to the ~600 b band detected with the *hslR* probe (Fig. S1A).

Supplemental Materials and Methods

Northern blots

BB7224 and BB7222 were grown aerobically at 30°C in LB medium. When an OD_{600} of 0.5 was reached, the cultures were shifted to 43°C to induce heat shock conditions. Disulfide stress was induced by addition of 1 mM diamide to the medium. For RNA extraction, 1.2 ml of culture were harvested on 600 µl ice cold killing buffer (20 mM NaN_3, 5 mM $MgCl_2$, 20 mM TRIS/HCl pH7.5) and the RNA was extracted using the RNEasy mini kit (Qiagen, Valencia, CA) according to the protocol provided by the manufacturer. Biotin-labeled probes to detect selected mRNAs (Table S2) were derived from double stranded DNA using the BrightStar Psoralen-Biotin Nonisotopic Labeling Kit (Ambion Inc., Austin, TX) according to the protocol provided. The DNA was obtained in a PCR using primers listed in Table S2 and chromosomal *E. coli* DNA as the template (3).

5 µg sample RNA was denatured at 55°C in 50% (v/v) formamide, 7% (v/v) formaldehyde, 5 mM sodium acetate, 1 mM EDTA, 20 mM MOPS pH 7.0, supplemented with 0.4 mg/ml bromophenol blue, 0.4 mg/ml xylene cyanol, 2.5% ficoll 400 and loaded onto a denaturing agarose gel (1.5% (w/v) agarose, 5 mM sodium acetate, 1 mM EDTA, 2.15% formaldehyde, 10 mM MOPS pH 7.0). The gel was run at 4 V/cm electrode distance. To analyze the quality of the RNA, to later verify the blotting efficiency and to normalize the northern signal to the total amount of RNA, the gel was stained with ethidium bromide staining solution (0.5% (w/v) ethidium bromide in 5 mM sodium acetate, 1 mM EDTA, 20 mM MOPS).

RNA was transferred via capillary blotting onto a Hybond N+™ nylon membrane (Amersham Biosciences, Piscataway, NJ) using 20 x SSC (0.3 M trisodium citrate, 3 M NaCl) as the transfer buffer. Then, the RNA was heat crosslinked to the membrane for 1 h at 80°C. The blots were placed into hybridization tubes and incubated in 5 ml Denhardt's buffer (5x SSC, 0.5% (w/v) SDS, 0.1% (w/v) ficoll 400, 0.1% (w/v) bovine serum albumine, 0.1% (w/v) polyvinylpyrrolidone) at 67.5°C for 1 h using a rotisserie oven. RNA was probed by adding 0.5 pmol of the relevant biotinylated probe to the prehybridization solution and by further incubation at 67.5°C overnight. Hybridization was detected using the BrightStar BioDetect Kit (Ambion Inc., Austin, TX) according to the manufacturer's protocol.

Supplemental Figures

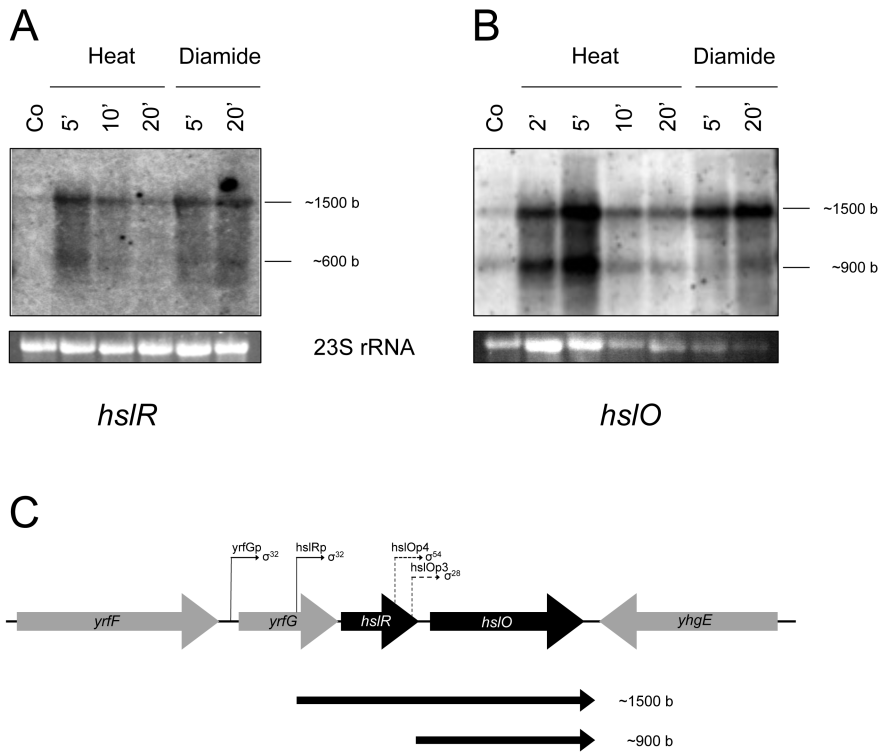

Figure S1: Expression of the *E. coli hslRO* operon. (**A**) *hslO* and (**B**) *hslR* are induced upon heat shock and diamide-induced disulfide stress in LB media. (**C**) Probes for *hslO* and *hslR* hybridize to a transcript of a length of about 1500 bases, indicating that *hslR* and *hslO* are cotranscribed and form a bicistronic operon. Smaller *hslR* and *hslO* transcripts of ~600 b (**A**) and ~ 900 b (**B**), respectively, indicate processing of the mRNA or additional transcription start sites.

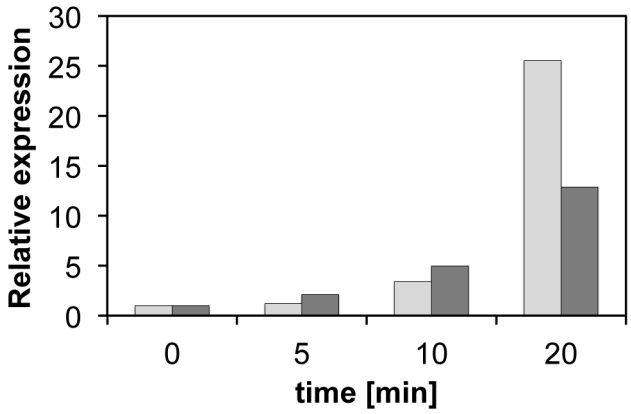

Figure S2: Normalization of *hslR* expression using *accD* and *phoP* as reference genes.

The expression of *hslR* upon disulfide stress was analyzed by normalization against expression of *accD* (light grey) and *phoP* (dark gray). Additional primers used for this experiment are listed in supplemental Table S3.

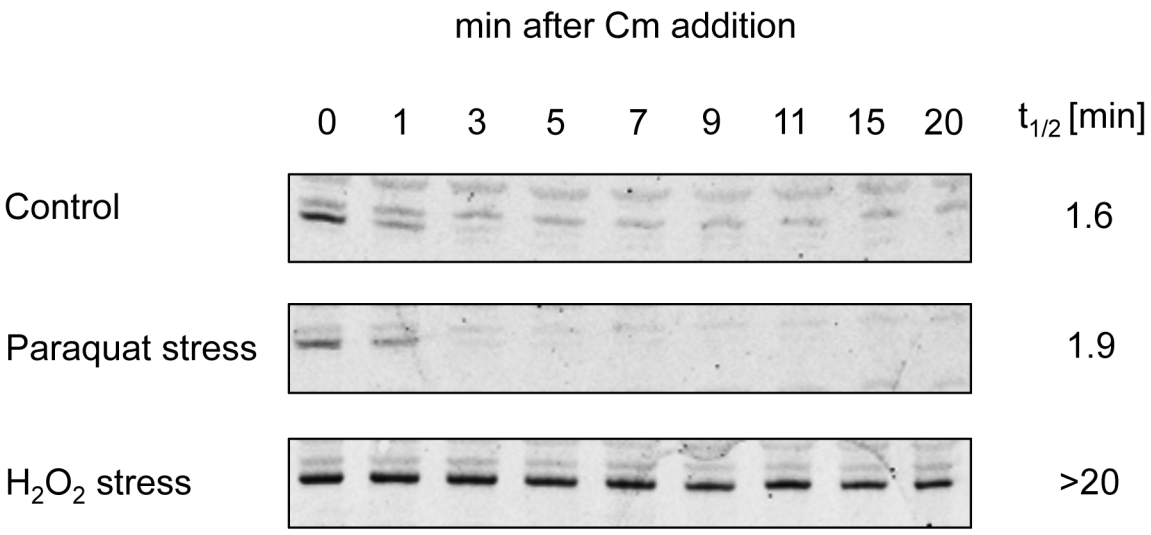

Figure S3: Stability of RpoH upon paraquat and H_2O_2 treatment. Expression of RpoH was induced by addition of 1 mM IPTG. Paraquat (250 µM) and H_2O_2 (4 mM) were added after 5 minutes and 15 minutes, respectively. Protein synthesis was blocked after a total of 25 minutes by the addition of 200 µg/mL chloramphenicol. Stability of RpoH was determined as described in Materials and Methods in the main manuscript.

Supplemental Tables

Table S1: Quality control of RNA and cDNA

	#1			#2		
RNA	**A260/280**	**A260/230**	**ng/µL**	**A260/280**	**A260/230**	**ng/µL**
WT control	2.11	2.12	207.6	2.12	2.17	225.5
WT heat 5 mins	2.13	2.23	239.6	2.04	2.15	242.8
WT heat 10 mins	2.10	2.19	315.1	2.12	2.10	324.6
WT heat 20 mins	2.12	2.25	411.2	2.11	2.24	329.5
WT diamide 5 mins	2.12	2.16	253.9	2.11	2.12	249.9
WT diamide 10 mins	2.11	2.24	265.1	2.12	2.08	214.2
WT diamide 20 mins	2.11	2.18	257.7	2.10	1.98	263.5
Δ*rpoH* control	2.12	2.04	143.6	2.11	2.08	234.5
Δ*rpoH* heat 5 mins	2.12	2.08	194.1	2.10	2.07	213.4
Δ*rpoH* heat 10 mins	2.12	1.98	204.8	2.10	2.07	256.3
Δ*rpoH* heat 20 mins	2.13	1.98	287.9	2.10	2.01	242.5
Δ*rpoH* diamide 5 mins	2.12	1.99	176.6	2.11	2.01	212.4
Δ*rpoH* diamide 10 mins	2.12	2.04	155.5	2.12	2.03	205.3
Δ*rpoH* diamide 20 mins	2.15	2.01	159.0	2.13	2.05	189.4
cDNA						
WT control	1.78	2.06	886.1	1.79	1.93	691.8
WT heat 5 mins	1.82	1.97	875.3	1.79	1.94	679.7
WT heat 10 mins	1.80	2.02	853.6	1.79	1.93	691.8
WT heat 20 mins	1.76	1.80	456.9	1.79	1.94	696.0
WT diamide 5 mins	1.76	2.12	791.4	1.80	1.93	729.6
WT diamide 10 mins	1.82	1.98	842.6	1.80	1.92	701.1
WT diamide 20 mins	1.77	2.05	814.0	1.78	1.90	699.5
Δ*rpoH* control	1.81	1.99	646.8	1.78	1.98	734.3

Δ*rpoH* heat 5 mins	1.81	2.01	679.6	1.78	1.99	673.2
Δ*rpoH* heat 10 mins	1.81	2.01	677.4	1.84	1.97	698.6
Δ*rpoH* heat 20 mins	1.80	2.03	713.8	1.79	2.05	710.4
Δ*rpoH* diamide 5 mins	1.82	2.01	645.4	1.85	2.03	712.3
Δ*rpoH* diamide 10 mins	1.81	1.98	631.6	1.81	1.86	731.3
Δ*rpoH* diamide 20 mins	1.83	1.97	697.8	1.81	2.01	659.5

76

77

Table S2 Primers used for Northern-Blot probe generation

Primer	**Sequence**
hslO forward	CATATGATTATGCCGCAACATGACC
hslO reverse	GGATCCTCATTAATGAACTTGCGGATC
hslR for	ATGAAAGAGAAACCTGCTG
hslR rev	TTATTCACTGTCGCCGTG
dnaJ for	ATGGCTAAGCAAGATTATTAC
dnaJ rev	TAGCGGGTCAGGTCGTC

Table S3 Primers used for RT-PCR control gene comparison

Primer	Sequence
phoP_F	ACATATACCGGATATTGCGATTGTC
phoP_R	CGGTTTAGTCACATAATCATCAGCA

Supplemental References

1. **Nonaka G**, **Blankschien M**, **Herman C**, **Gross CA**, **Rhodius VA**. 2006. Regulon and promoter analysis of the *E. coli* heat-shock factor, σ^{32}, reveals a multifaceted cellular response to heat stress. Genes Dev. **20**:1776–1789.
2. **Salgado H**, **Peralta-Gil M**, **Gama-Castro S**, **Santos-Zavaleta A**, **Muñiz-Rascado L**, **García-Sotelo JS**, **Weiss V**, **Solano-Lira H**, **Martínez-Flores I**, **Medina-Rivera A**, **Salgado-Osorio G**, **Alquicira-Hernández S**, **Alquicira-Hernández K**, **López-Fuentes A**, **Porrón-Sotelo L**, **Huerta AM**, **Bonavides-Martínez C**, **Balderas-Martínez YI**, **Pannier L**, **Olvera M**, **Labastida A**, **Jimenez-Jacinto V**, **Vega-Alvarado L**, **Del Moral-Chávez V**, **Hernández-Alvarez A**, **Morett E**, **Collado-Vides J**. 2013. RegulonDB v8.0: omics data sets, evolutionary conservation, regulatory phrases, cross-validated gold standards and more. Nucleic Acids Res. **41**:D203–D213.
3. **Rozen S**, **Skaletsky H**. 2000. Primer3 on the WWW for general users and for biologist programmers. Methods Mol. Biol. **132**:365–386.