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NOTES

Genome-Wide Transcriptional Analysis of the Cold Shock Response in Wild-Type and Cold-Sensitive, Quadruple-*csp*-Deletion Strains of *Escherichia coli*

Sangita Phadtare* and Masayori Inouye

Department of Biochemistry, Robert Wood Johnson Medical School, Piscataway, New Jersey

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A DNA microarray-based global transcript profiling of *Escherichia coli* in response to cold shock showed that in addition to the known cold shock-inducible genes, new genes such as the flagellar operon, those encoding proteins involved in sugar transport and metabolism, and remarkably, genes encoding certain heat shock proteins are induced by cold shock. In the light of strong reduction in metabolic activity of the cell after temperature downshift, the induction of sugar metabolism machinery is unexpected. The deletion of four *csp*s (*cspA*, *cspB*, *cspG*, and *cspE*) affected cold shock induction of mostly those genes that are transiently induced in the acclimation phase, emphasizing that CspA homologues are essential in the acclimation phase. Relevance of these findings with respect to the known RNA chaperone function of CspA homologues is discussed.

The cold shock response is a physiological response of living cells to temperature downshift (12) and has been studied in detail using *Escherichia coli* and *Bacillus subtilis* as model systems (for a review, see references 5, 21, 26, and 32). When an exponentially growing culture of *E. coli* is shifted from 37 to 15°C, an acclimation phase (lag period of cell growth) characterized by transient dramatic induction of cold shock proteins against a severe inhibition of general protein synthesis precedes the resumption of growth. Out of the nine CspA homologues of *E. coli*, only CspA, CspB, CspG, and CspI are cold shock inducible (6, 17, 20, 31). Interestingly, double or triple deletions of genes encoding cold shock-inducible CspA homologues do not result in cold sensitivity. In a triple-deletion strain, the Δ*cspA* Δ*cspB* Δ*cspG* strain, CspE that is normally produced at 37°C is overproduced at low temperatures (33). This observation suggests that the functions of the CspA family members may overlap and they are able to substitute for each other during cold acclimation. Indeed a quadruple-deletion strain (the Δ*cspA* Δ*cspB* Δ*cspG* Δ*cspE* strain) of *E. coli* exhibits cold sensitivity at 15°C, which can be complemented by overproduction of any one of the CspA homologues except CspD (33).

In spite of a wealth of knowledge accumulated in recent years, the cold shock response is not fully elucidated. The proteomic approaches that so far have been extremely useful in identification of many cold shock-induced proteins do have certain limitations: (i) not all the proteins can be resolved well on two-dimensional gel electrophoresis, and (ii) identification of proteins from the gel may sometimes be cumbersome. To overcome these shortcomings, in the present study, we carried out analysis of global cold shock gene expression profiles of an *E. coli* wild-type and cold-sensitive quadruple-deletion strain. Our main objectives were (i) to identify the *E. coli* open reading frames that exhibit significant increase or decrease in mRNA abundance caused by the temperature downshift and (ii) to explore the effect of deletion of four *csp* genes that leads to cold sensitivity. In brief, the *E. coli* JM83 strain [F^- *ara*Δ(*lac-proAB*) *rpsL*(Str^r)] (35) (considered the wild-type strain in this study) was grown in Luria broth (LB). The cells grown overnight in LB medium at 37°C were diluted into fresh LB medium. Cells were grown at 37°C to exponential phase (optical density at 600 nm [OD_{600}] of 0.8), and part of the cell culture was harvested and used as a control. Aliquots of the cells were transferred to a prechilled LB medium at 15°C, and the cells were harvested after 1 and 5 h of cold shock. The OD_{600} did not increase after 1 h of cold shock, while after 5 h of cold shock, it was 1.2. The 37°C controls were of corresponding OD_{600} values. For studies involving the quadruple-deletion strain, the wild-type and the deletion strain were grown at 37°C and subsequently cold shocked for 1 h as described above. Note that in the studies involving the quadruple-deletion strain, both the wild-type and the deletion cells are cold shocked at 15°C for 1 h and compared with each other. This enables us to directly single out the genes that were differentially expressed as a result of *csp* deletion upon cold shock. The RNA extraction and hybridization and DNA array analysis were carried out as described previously (24). The cell density of all samples used was the same; thus, the changes seen in the microarray were not substantially influenced by the difference in cell densities. Genes whose expression levels differed by a ratio of at least 4 after cold shock were considered. From replicates, we estimate that the chance random fluctuations giving rise to a fourfold up- or down-modulation is less than 0.14%, corresponding to a confidence interval of 99.86%. Thus, the chosen fourfold cutoff value is rather stringent and

* Corresponding author. Mailing address: Department of Biochemistry, Robert Wood Johnson Medical School, 675 Hoes Ln., Piscataway, NJ 08854. Phone: (732) 235-4116. Fax: (732) 235-4559. E-mail: phadtasa@umdnj.edu.

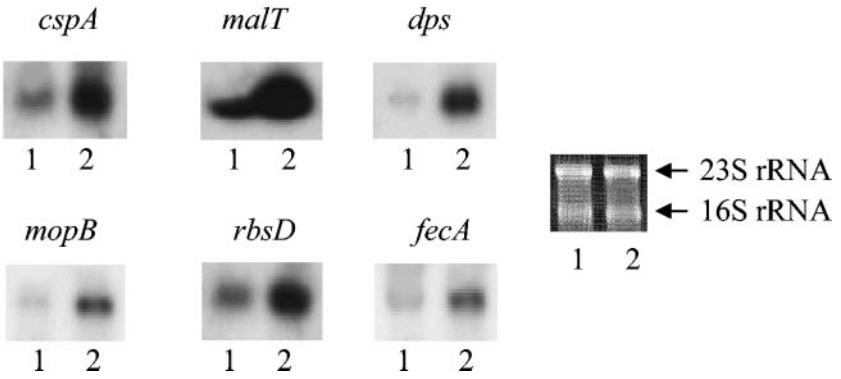

FIG. 1. Effect of cold shock on the levels of mRNAs. Total RNA was extracted by the hot phenol method as described in the text, and Northern blot analysis was carried out with deoxyoligonucleotides corresponding to *cspA*, *malT*, *dps*, *mopB*, *rbsD*, and *fecA.* Lanes 1 and 2 in each case except *fecA* represent mRNAs isolated from control (37°C) and cold-shocked (1 h) wild-type cells, respectively. In the case of *fecA*, lanes 1 and 2 represent mRNAs isolated from cold-shocked (1 h) wild-type and quadruple-deletion cells, respectively. The positions of the transcripts were determined using as reference ribosomal RNAs. Bands corresponding to 23S and 16S rRNAs were visualized by ethidium bromide staining of the gel.

the modulation of expression beyond the cutoff is highly statistically significant. In some cases, genes belonging to the same operon or category were considered even if the ratios did not adhere to these specified values. Ratios above 1 indicate induction and below 1 indicate repression. Ratios averaged from three independent of sets of experiments are shown with standard deviation values.

The result of the microarray analysis was confirmed by Northern blot analysis. The genes chosen for these experiments represent the important groups changed by the cold shock treatment, such as *cspA* and those encoding proteins involved in sugar metabolism, molecular chaperones, and iron metabolism. The deoxyoligonucleotides used for detection of *cspA* and *dps* were described previously (22, 24, 34). The deoxyoligonucleotides used for detection of *malT* (4) (accession number M13585), *mopB* (19), *rbsD* (3), and *fecA* (29) correspond to the region from codons 13 to 6 of *malT* and *mopB*, 21 to 14 of *rbsD*, and 13 to 8 of *fecA*. The Northern blot analysis was carried out as described previously (30). The results are shown in Fig. 1. These results are consistent with the microarray data.

The reliability of the present data was confirmed by (i) reproducible values obtained in multiple, independent experiments, (ii) induction of many known cold shock-inducible genes, and (iii) confirmation of levels of some of the significantly affected genes by Northern blot analysis. Our data did not show mRNA abundance of certain genes known to be cold shock-inducible, for example, genes belonging to the *nusA-pnp* operon, such as *nusA*, *infB*, *rbfA*, and *pnp*. The reason for this is not known at present; however, this observation is similar to that from a recent report by Polissi et al. (27), in which the discrepancy was attributed to differential stability of the 3′ end and the entire mRNA transcripts.

The genes affected by cold shock are grouped as those that (i) are transiently induced immediately following the cold shock in the acclimation phase (Table 1), (ii) show transient repression upon cold shock (Table 2), (iii) show prolonged induction beyond the acclimation phase (Table 3), and (iv) show prolonged repression upon cold shock.

Genes transiently induced and repressed in the acclimation phase upon cold shock. The present analysis showed transient induction of a number of known cold shock-inducible genes, for example, *cspA*, *cspB*, *cspG*, *cspI*, *otsA*, *otsB*, and *ppiA* (Table 1). Other known genes such as *gyrA* (twofold), *infA* (twofold), *infC* (2.8-fold), and *recA* (threefold) were also induced. New genes shown by the present analysis to be cold shock-inducible in the acclimation phase include the following: (i) transport or metabolism of sugars (fructose, glucose, glycerol, maltose, mannose, ribose, and xylose) and (ii) molecular chaperones (*mopA* and *mopB*, encoding GroEL and GroES, respectively, *htpG*, and *ppiA*). Deletion of four *csp* genes led to repression of cold shock induction of all these genes (Table 1). Although cold shock response is characterized by strong repression of the major metabolic activity of the cell, the present study showed induction of several new genes after the temperature downshift. Transport and metabolism systems for sugars deserve special mention in this aspect. Cold shock caused induction of *otsA* (trehalose-6-phosphate synthase) and *otsB* (trehalose-6-phosphate phosphatase) (Table 1), consistent with the previously reported possible protective effect of this sugar upon cold shock (14). However, such a protective effect is not known for sugars such as ribose or mannose that were induced in the present system, and this induction could simply be a manifestation of the cell gearing up for the low-temperature-adapted metabolism. It should be noted that recently, cold stress accumulation and protective effect of maltose in plants was reported (16). It is interesting that cold shock induction of mannose and maltose transport systems was prominently repressed in the quadruple deletion that has significantly prolonged (4 h) lag period as opposed to the 1-h lag period of the wild-type strain (33) after the temperature downshift. This suggests that cold shock induction of these genes is indeed relevant for the cold acclimation of the cells.

As the cold shock response of the quadruple-deletion strain was severely affected, many genes repressed in the wild type upon cold shock were further repressed in the deletion strain. In addition to the genes listed in Table 1, the genes repressed in the quadruple-deletion strain included those involved in transport (ATP synthase, DctA protein, DsdX permease, fatty acid transport protein, maltoporin precursor, OmpF, OmpX, thiamine-binding protein precursor, and tryptophan permease) and a number of genes involved in cellular metabolism (especially amino acids and sugars).

TABLE 1. Genes transiently induced upon cold shock

Gene name and role	Gene product and/or function	Wild-type 15°C/37°C ratio at 1 h	Wild-type 15°C/37°C ratio at 5 h	Mutant/wild-type ratio (15°C, 1 h)
Genes involved in membrane synthesis/function				
aer	Aerotaxis receptor	9.8 ± 0.14	4.8 ± 0.4	
atoE	Short-chain fatty acid transporter	6.5 ± 0.5	3 ± 0.5	0.22 ± 0.08
dctA	DctA protein	7.8 ± 1.07	1.75 ± 0.6	0.1 ± 0
fabB	3-Oxoacyl-[acyl-carrier protein] synthase I	13 ± 1.1	2.6 ± 0.35	
glnH	Glutamine-binding protein precursor	6.1 ± 0.73	1 ± 0.1	
malE, *-F*, *-K*, *-M*	Maltose transport proteins	9.0–10 ± 0.5	0.5–2.7 ± 0.1	0.03–0.08 ± 0.01
manY	Phosphotransferase system enzyme II	8.9 ± 0.28	1.9 ± 0.2	0.13 ± 0.02
manZ	PTS system, mannose-specific IID component	6.9 ± 0.39	0.3 ± 0.01	0.14 ± 0.03
nupC	Nucleoside permease NupC	9.3 ± 0.015	1.2 ± 0.09	
rbsA-D	Ribose transport proteins	5.0–10 ± 0.8	1.5–2 ± 0.2	0.05–0.09 ± 0.02
sanA	SanA protein	4.3 ± 0.28	2.1 ± 0.5	
trg	Methyl-accepting chemotaxis protein III	9.2 ± 0.8	4.1 ± 0.6	
xylF	D-Xylose-binding periplasmic protein precursor	20 ± 2.1	3.5 ± 0.4	0.24 ± 0.03
ybeJ	Amino acid ABC transporter binding protein	6.8 ± 0.08	1.8 ± 0.5	
Genes involved in cell metabolism				
adhE	Alcohol dehydrogenase	18.8 ± 0.48	3.2 ± 0.45	
agp	Glucose-1-phosphatase precursor	16 ± 2	2.9 ± 0.3	0.21 ± 0.06
aldA	Aldehyde dehydrogenase	15 ± 1.5	0.15 ± 0.01	0.15 ± 0.01
aphA	Acid phosphatase	16.2 ± 0.44	2.1 ± 26	0.12 ± 0.005
aspA	Aspartate ammonia-lyase	20 ± 3	7.3 ± 0.8	0.13 ± 0.005
bfr	Bacterioferritin	5 ± 1	1 ± 0.1	
carA	Carbamoyl-phosphate synthase small chain	8.5 ± 0.15	0.9 ± 0.1	
cfa	Cyclopropane fatty acid synthase	8.2 ± 1.16	3.7 ± 0.9	
cpdB	Cyclic nucleotide 2'phosphodiesterase	10 ± 2	1.6 ± 0.15	0.12 ± 0.03
cysK	Cysteine synthase	7 ± 1.27	0.6 ± 0.025	
deoA	Thymidine phosphorylase	21.8 ± 1.02	3.6 ± 0.33	
fpr	Ferredoxin-$NADP^+$ reductase	4.5 ± 0.45	1.6 ± 0.31	
fruB	PTS system, fructose-specific IIA/FPR component	7 ± 0.16	0.8 ± 0.2	0.16 ± 0.03
fruK	1-Phosphofructokinase (fructose 1-phosphate kinase)	6.5 ± 0.75	0.7 ± 0.2	0.18 ± 0.015
fucU	Fucose operon FucU protein	6.3 ± 0.87	2.7 ± 0.22	
fumA	Fumarate hydratase	9.3 ± 0.79	1 ± 0.08	
gapA	Glyceraldehyde-3-phosphate dehydrogenase	13.5 ± 1.2	2.3 ± 0.3	0.2 ± 0.01
glpK	Glycerol kinase	12.8 ± 0.55	1 ± 0.02	
lipA	Lipoic acid synthetase (lip-syn)	4.2 ± 0.02	1.7 ± 0.25	
malP	Maltodextrin phosphorylase	20 ± 2.5	4 ± 0.75	
malQ	4-Alpha-glucanotransferase	6.3 ± 0.46	3 ± 0.075	
malT	MalT regulatory protein	12 ± 0.72	4.2 ± 0.22	
manX	Phosphotransferase system enzyme II	11.4 ± 0.7	2.4 ± 0.03	0.13 ± 0.03
mdh	Malate dehydrogenase	3.2 ± 0.3	0.28 ± 0	0.24 ± 0.05
nrdD	Oxygen-sensitive ribonucleoside-triphosphate reductase	15.2 ± 1.58	1.8 ± 0.22	
otsA	Tehalose-6-phosphate synthase	4.3 ± 0.12	1 ± 0.29	
otsB	Trehalose-phosphatase	2.7 ± 0.19	0.7 ± 0.15	
pgi	Glucose-6-phosphate isomerase	6.1 ± 0.15	1.7 ± 0.15	0.19 ± 0.025
poxB	Pyruvate oxidase	3.6 ± 0.6	0.18 ± 0.02	0.25 ± 0.04
pykA	Pyruvate kinase	7.4 ± 0.14	0.75 ± 0.16	0.16 ± 0
rbsK	Ribokinase	5.1 ± 0.34	2.9 ± 0.37	0.19 ± 0.04
srlA	Phosphoenolpyruvate-carbohydrate phosphotransferase system, glucitol/sorbitol-specific IIBC component	9.3 ± 1.3	2.7 ± 0	
srlB	Phosphotransferase system enzyme II	4.4 ± 0.22	1.5 ± 0.3	
srlD	Sorbitol-6-phosphate 2-dehydrogenase	5.1 ± 0.23	1.5 ± 0.02	
srlR	Glucitol operon repressor	5.5 ± 0.06	2.6 ± 0.4	
treB	Phosphotransferase system trehalose permease	6 ± 1	0.7 ± 0.01	
treC	Trehalose-6-phosphate hydrolase	6 ± 1.1	0.45 ± 0.05	
udp	Uridine phosphorylase	10 ± 1.5	0.62 ± 0.02	0.08 ± 0
ybeK	Pyrimidine-specific nucleoside hydrolase	10.2 ± 0.55	1.6 ± 0.2	
Genes encoding proteins with diverse functions				
cspA	CspA	4 ± 0.3	1 ± 0.1	
cspB	CspB	9 ± 0.4	1 ± 0.1	
cspG	CspG	6 ± 0.5	0.8 ± 0.07	
cspI	CspI	2 ± 0.03	0.7 ± 0.05	
dps	DNA-binding protein Dps	10 ± 1.3	0.9 ± 0.015	0.05 ± 0.01
grxB	Glutaredoxin 2	4.7 ± 0.4	1 ± 0.06	
hns	DNA-binding protein H-NS	4.8 ± 0.8	2.4 ± 0.5	0.5 ± 0.1
hobH	DNA binding protein, replication-origin specific	10 ± 2	2 ± 0.35	0.15 ± 0.07
htpG	Heat shock protein C62.5	4.5 ± 0.5	1.6 ± 0.4	0.27 ± 0.005
kbl	2-Amino-3-ketobutyrate coenzyme A ligase	4.8 ± 0.24	1.8 ± 0.2	
mdaA	Modulator of drug activity A	11.6 ± 0.06	4 ± 0.9	
mlc	Making large colonies protein	6.8 ± 0.29	2.5 ± 0.67	
mopA	GroEL protein	9.3 ± 0.88	1.3 ± 0.21	0.13 ± 0.01
mopB	GroES protein	8 ± 1	0.9 ± 0.02	0.18 ± 0.04
ppiA	Peptidyl-prolyl-cis-trans-isomerase A precursor	4.6 ± 0.51	1.7 ± 0.24	0.22 ± 0.06
rimJ	Ribosomal-protein-alanine acetyltransferase	6.5 ± 0.74	2.7 ± 0.03	
sseA	Putative thiosulfate sulfurtransferase	7.6 ± 1.2	1.8 ± 0.15	

TABLE 2. Genes showing transient repression upon cold shock in the wild-type strain

Gene name and role	Gene product and/or function	15°C/37°C ratio at 1 h	15°C/37°C ratio at 5 h
Genes involved in membrane synthesis/function			
fecA	Iron(III) dicitrate transport protein FecA precursor	0.02 ± 0.005	0.22 ± 0.001
fecB	Iron(III) dicitrate-binding periplasmic protein precursor	0.04 ± 0.01	0.3 ± 0.08
fecC	FecC protein	0.02 ± 0	0.9 ± 0.2
fecE	Membrane-bound iron (III) dicitrate transport protein	0.01 ± 0.005	0.9 ± 0.07
fepC	Ferric enterobactin transport protein FepC	0.14 ± 0.03	0.7 ± 0.06
fimD	FimD protein	0.29 ± 0.04	1.9 ± 0.13
kgtP	Alpha-ketoglutarate permease	0.3 ± 0.02	0.45 ± 0.01
lgt	Prolipoprotein diacylglyceryl transferase	0.25 ± 0.005	0.54 ± 0.12
lolA	Outer membrane lipoproteins carrier protein precursor	0.27 ± 0.005	0.6 ± 0.04
msbA	MsbA protein	0.26 ± 0.015	0.6 ± 0.02
nlpD	Lipoprotein D precursor	0.7 ± 0.02	0.26 ± 0.02
oppB	Oligopeptide transport system permease protein	0.31 ± 0	1.1 ± 0.1
oppC	Oligopeptide permease membrane protein	0.27 ± 0.01	1.5 ± 0.2
plsX	PlsX protein	0.16 ± 0.03	1 ± 0.005
potA	Spermidine/putrescine transport protein A	0.22 ± 0.01	0.45 ± 0.08
potB	Spermidine/putrescine transport system permease protein PotB	0.12 ± 0.01	0.31 ± 0.045
potC	Spermidine/putrescine transport system permease protein PotC	0.19 ± 0.025	0.31 ± 0.015
proP	Proline/betaine transport protein	0.17 ± 0.01	0.48 ± 0.01
proV	Glycine betaine/I-proline transport ATP-binding protein ProV	0.01 ± 0.005	0.06 ± 0.005
proW	Glycine betaine/proline transport system protein prow	0.04 ± 0	0.07 ± 0.015
proX	Glycine betaine-binding periplasmic protein precursor	0.08 ± 0.01	0.11 ± 0.01
secG	P12 cytoplasmic membrane protein	0.3 ± 0.005	0.57 ± 0.05
tolA	TolA protein	0.29 ± 0.025	1.2 ± 0.2
trkH	TrkH protein	0.33 ± 0	1.3 ± 0.2
wzxE	Lipopolysaccharide biosynthesis protein	0.19 ± 0	0.88 ± 0.03
Genes involved in cell metabolism			
aceB	Malate synthase A (Msa)	0.04 ± 0	0.1 ± 0.005
adhC	Formaldehyde dehydrogenase (glutathione)	0.26 ± 0.035	0.45 ± 0.045
argB	Acetylglutamate kinase	0.18 ± 0.01	0.75 ± 0.01
argC	*N*-acetyl-gamma-glutamyl-phosphate reductase	0.12 ± 0.04	3 ± 0.4
argD	Acetylomithine aminotransferase	0.14 ± 0.02	0.3 ± 0.1
argG	Argininosuccinate synthase	0.15 ± 0.04	1.3 ± 0.15
argH	Argininosuccinate lyase	0.25 ± 0.08	0.4 ± 0.05
aroA	3-Phosphoshikimate 1-carboxyvinyltransferase	0.2 ± 0.01	0.45 ± 0.07
aroB	3-Dehydroquinate synthase	0.3 ± 0.015	1.2 ± 0.24
cmk	Cytidylate kinase (cytidine monophosphate kinase)	0.24 ± 0.02	0.4 ± 0.01
ddg	Ddg protein	0.1 ± 0.01	1.4 ± 0.01
fdhE	FdhE protein	0.27 ± 0	0.7 ± 0.6
folP	Dihydropteroate synthase	0.29 ± 0.03	0.5 ± 0.02
glcF	Glycolate oxidase iron-sulfur subunit	0.07 ± 0.005	1.7 ± 0.27
gltB	Glutamate synthase (NADPH) large chain precursor	0.12 ± 0.02	0.5 ± 0.005
hisF	Cyclase HisF	0.3 ± 0.02	0.8 ± 0.2
iclR	Repressor protein IclR	0.08 ± 0.01	0.56 ± 0.005
phoH	PhoH protein	0.5 ± 0.07	0.08 ± 0.005
psd	Phosphatidylserine decarboxylase precursor	0.32 ± 0	0.43 ± 0.01
rffH	Glucose-1-phosphate thymidylyltransferase	0.2 ± 0.01	0.55 ± 0.05
thil	Thiamin biosynthesis protein	0.27 ± 0.09	1.7 ± 0.5
thyA	Thymidylate synthase	0.28 ± 0	0.45 ± 0.02
truA	Pseudouridylate synthase I	0.31 ± 0.01	0.7 ± 0.08
Genes encoding proteins with diverse functions			
dedA	DedA protein	0.29 ± 0.02	0.45 ± 0.07
dedE	DedE protein	0.3 ± 0.025	0.5 ± 0.09
dinG	Probable ATP-dependent helicase DinG	0.24 ± 0.003	1.4 ± 0.13
dnaG	DNA primase	0.2 ± 0.015	1.1 ± 0.13
fkpA	FkpA protein	0.29 ± 0.005	0.47 ± 0.005
fldB	Flavodoxin	0.32 ± 0.005	1.26 ± 0
ftsH	Cell division protein FtsH, protease	0.25 ± 0.01	0.9 ± 0.06
ftsJ/rrmJ	Cell division protein, 23S rRNA methyltransferase	0.29 ± 0.015	1.2 ± 0.12
ftsK	Cell division protein FtsK	0.2 ± 0.01	0.9 ± 0.07
gidA	GidA protein	0.24 ± 0.01	0.8 ± 0.09
hflC	HflC protein	0.22 ± 0.02	0.54 ± 0
hflK	HflK protein	0.25 ± 0.01	0.7 ± 0.06
holA	DNA-directed DNA polymerase III delta chain	0.31 ± 0.005	0.7 ± 0.12
hscA	Heat shock cognate protein 66	0.09 ± 0.01	0.7 ± 0.13
ksgA	Dimethyladenosine transferase	0.24 ± 0.04	0.75 ± 0.07
lepA	GTP-binding protein LepA	0.28 ± 0.02	0.5 ± 0.03
mrcA	Penicillin-binding protein 1a (pbp-1a)	0.28 ± 0.02	1.3 ± 0.01
mrdB	Rod shape-determining protein MrdB	0.18 ± 0.02	0.74 ± 0.02
pepB	Peptidase B	0.17 ± 0	0.3 ± 0.06
priA	Primosomal replication factor Y	0.27 ± 0.015	1.1 ± 0.3
recB	Exodeoxyribonuclease V 135-kDa polypeptide	0.22 ± 0.01	1.2 ± 0.03

Continued on following page

TABLE 2—*Continued*

Gene name and role	Gene product and/or function	15 °C/37°C ratio at 1 h	15 °C/37°C ratio at 5 h
recC	Exodeoxyribonuclease V 125-kDa polypeptide	0.32 ± 0.01	0.9 ± 0.06
recJ	Single-stranded-DNA-specific exonuclease	0.3 ± 0.02	1.2 ± 0.17
rimM	16S rRNA processing protein	0.16 ± 0.005	0.47 ± 0
mhA	Ribonuclease HI	0.26 ± 0.02	0.85 ± 0.15
rpoA	DNA-directed RNA polymerase alpha chain	0.23 ± 0.03	0.6 ± 0.03
rpoB	DNA-directed RNA polymerase beta chain	0.18 ± 0.005	0.4 ± 0.02
rrmA	rRNA (guanine-N1)-methyltransferase	0.25 ± 0.045	0.15 ± 0.025
rtn	Rtn protein	0.28 ± 0.01	1.65 ± 0.13
sdiA	SdiA regulatory protein	0.26 ± 0.025	0.44 ± 0.005
sodA	Superoxide dismutase	0.26 ± 0.01	0.07 ± 0.01
tnpR	Resolvase	0.08 ± 0.005	0.4 ± 0.1
topA	DNA topoisomerase I	0.23 ± 0.025	0.35 ± 0.04
trmD	tRNA (guanine-n1)-methyltransferase	0.17 ± 0.005	0.42 ± 0.02

On the other hand, a few genes were transiently induced in the quadruple-deletion strain compared with the wild-type strain, and these prominently constitute the genes encoding proteins involved in transport of iron, such as *exbD*, *fecA* to *fecD*, *fepC*, and *fhuA* and *fhuF* (Table 4). It is not clear why deletion of *csp* genes should result in induction of iron transport. It is noteworthy that these genes were repressed in the wild-type cells upon temperature downshift. In fact, judging from Table 4, a very divergent group of genes was induced by deletion of the four *csp* genes.

In addition to the genes listed in Table 2, most of the genes encoding ribosomal L proteins showed transient reduced levels

TABLE 3. Genes showing prolonged induction upon cold shock

Gene name and role	Gene product and/or function	Wild-type 15°C/37°C ratio at 1 h	Wild-type 15°C/37°C ratio at 5 h	Mutant/ wild-type ratio (15°C, 1 h)
Genes involved in membrane synthesis/function				
cheW	Chemotaxis protein CheW, adapter protein	3.5 ± 0.05	5.4 ± 0.01	
cheY	Chemotaxis protein CheY	4.3 ± 0.09	3.6 ± 0.51	
dcuA	Anaerobic C4-dicarboxylate transporter DcuA	7.4 ± 0.36	7.3 ± 0.7	
dmsC	Dimethylsulfoxide reductase chain C	4.3 ± 0.09	7 ± 1	
flg and fli	Flagellar proteins	2.0–12 ± 0.5	4.0–13 ± 1	0.01–0.1 ± 0
frdA	Fumarate reductase flavoprotein subunit	20 ± 0.5	15 ± 1	0.2 ± 0.02
frdB	Fumarate reductase iron-sulfur protein	17 ± 0.9	11 ± 0.9	
frdD	Fumarate reductase, 13-kDa membrane anchor protein	6 ± 0.55	3.5 ± 0.7	
glpQ	Glycerophosphodiester phosphodiesterase	4.3 ± 0.13	5.5 ± 1	
glpT	Glycerol-3-phosphate transport protein	7.8 ± 0.33	6.1 ± 0.36	
hybA	HybA protein	8.4 ± 0.92	8 ± 1	
hybB	HybB protein	4.6 ± 0.23	11 ± 1.5	
hybC	HybC protein	4.5 ± 0.13	6 ± 0.6	
hypB	Hydrogenase isoenzymes formation protein	17.8 ± 1.37	17 ± 1.5	
hypE	HypE protein	5.3 ± 0.12	13 ± 1.3	
nupG	Nucleoside-transporting protein NupG	18.6 ± 2.28	15 ± 1.4	
ompC	Outer membrane protein C precursor	5 ± 0.1	4 ± 0.08	0.24 ± 0.02
ompT	Proteinase VII precursor	19.7 ± 1.25	57 ± 6	
tap	Methyl-accepting chemotaxis protein II	4.2 ± 0.12	4.8 ± 0.4	
tar	Methyl-accepting chemotaxis protein II	11.1 ± 0.85	7.8 ± 1	
Genes involved in cell metabolism				
ackA	Acetate kinase	4.2 ± 0	5.5 ± 0.02	
ansB	Asparaginase	10.8 ± 0.16	25.3 ± 3	
asnB	Asparagine synthase	5.1 ± 0.34	6 ± 2	
cydA	Cytochrome *d* ubiquinol oxidase subunit I	3.8 ± 0.35	5.7 ± 0.9	
ftn	Ferritin	13 ± 1	13 ± 0.9	
fumB	Fumarate hydratase	9.6 ± 1.34	9.6 ± 1.5	
glpB	Glycerol-3-phosphate dehydrogenase	20.3 ± 1.43	14.8 ± 0.28	
glpC	Glycerol-3-phosphate dehydrogenase	27.4 ± 3.7	14.1 ± 2.5	
pflB	Formate C-acetyltransferase	5.6 ± 0.46	5.5 ± 1.5	
wrbA	Trp repressor binding protein	15 ± 2	8 ± 1	
Genes encoding proteins with diverse functions				
clpB	ClpB protein (heat shock protein)	10.4 ± 0.4	9.4 ± 0.39	
gst	Glutathione transferase	7.4 ± 0.36	5.1 ± 1	
hsdR	Type I restriction enzyme EcoKI R protein	9 ± 1	9.7 ± 0.92	
katG	Catalase HPI	17 ± 0.06	12.3 ± 1	
speG	Spermidine n1-acetyltransferase	3 ± 0.25	5 ± 0.38	

TABLE 4. Genes induced by cold shock in the quadruple-deletion strain

Gene name and role	Gene product and/or function	Mutant/wild type ratio (15°C, 1 h)
Genes involved in membrane synthesis/function		
exbD	ExbD protein	4.4 ± 0.2
fecA	Iron(III) dicitrate transport protein FecA precursor	4 ± 0.21
fecB	Iron(III) dicitrate-binding periplasmic protein precursor	12.5 ± 1.1
fecC	FecC protein	4 ± 0.2
fecD	Iron(III) dicitrate transport system permease protein	5.2 ± 0.18
fecE	Membrane-bound iron(III) dicitrate transport protein	5.8 ± 0.4
fepC	Ferric enterobactin transport protein FepC	3.2 ± 0.08
fhuA	Ferrichrome-iron receptor precursor	3.9 ± 0.4
fhuF	Ferric hydroxamate transport protein	7 ± 0.2
livK	Leucine transport protein LivK precursor	4.8 ± 0.4
Genes involved in cell metabolism		
acpS	Holo-[acyl-carrier protein] synthase	3 ± 0.1
aroE	Shikimate 5-dehydrogenase	4.3 ± 0.25
aroG	Phospho-2-dehydro-3-deoxyheptonate aldolase	4.2 ± 0.3
cobU	Cobinamide kinase	3.9 ± 0.2
ddg	Ddg protein	5.4 ± 0.4
gltD	Glutamate synthase	5.5 ± 0.18
gltF	GltF protein	4.8 ± 0.4
Genes encoding proteins with diverse functions		
creA	CreA protein	5.1 ± 0.05
deaD	ATP-dependent RNA helicase	5 ± 1
fimE	Type 1 fimbriae regulatory protein FimE	5.5 ± 1
fimF	FimF protein	7 ± 1.1
hha	Hha protein	3.6 ± 0.5
intA	Prophage cp4-57 integrase	3.5 ± 0.19
rhlE	Putative ATP-dependent RNA helicase RhIE	4.1 ± 0.4
rpoE	RNA polymerase sigma-E factor (sigma-24)	4.6 ± 0.44
rseB	RseB protein	3.2 ± 0.5
rseC	Sigma-E factor regulatory protein	5.5 ± 0.4
sdiA	SdiA regulatory protein	3.5 ± 0.12
tnpR	Resolvase	5.1 ± 0.6

during acclimation phase in the wild-type strain, although in the latter the effect was not severe (approximately two- to threefold) and their synthesis recovered after continued growth at 15°C for 5 h. This result is consistent with cold shock two-dimensional gel electrophoresis data published for *E. coli* (26).

In addition, the products of a number of genes, such as *ybdQ*, *ycfP*, *ydaA*, *ydhO*, *yeaA*, *yedU*, *yeeX*, *yefI*, *yfbU*, *yfiA*, *yfjL*, *yggG*, *ygjR*, *yhbT*, *yhjH*, *yieP*, *yqeB*, *yqhD*, and *yzzQ*, increased significantly, although the products have not been assigned any functions. On the other hand, *priB*, *yaeG*, *yafK*, *ybiR*, *ybiT*, *ycaJ*, *ycaO*, *yccA*, *yceD*, *yceP*, *ycfC*, *ycfV*, *ycfX*, *ycgE*, *ydgR*, *ydiU*, *yedA*, *yedI*, *yfcA*, *yfK*, *yfgL*, *yfgM*, *yfiH*, *yfiR*, *ygdE*, *yggN*, *yhaD*, *yhaE*, *yhaF*, *yhaU*, *yhbM*, *yheQ*, *yhiN*, *yjgP*, *yqgE*, *yqgF*, and *yrbE* were repressed.

Genes showing prolonged induction and repression upon cold shock. Genes encoding flagellar proteins were induced and maintained at high levels even after 5 h at 15°C in the wild-type strain and were down-regulated in the quadruple-deletion strain. Spermidine acetyltransferase encoded by *speG* is required to prevent spermidine toxicity at low temperatures in *E. coli* (18). Our DNA microarray analysis showed a steady increase in *speG* levels from three- to fivefold at 1 to 5 h after temperature downshift (Table 3). On the other hand, genes such as *tas* (Tas protein), *artP* (ArtP protein), those mainly involved in amino acid and nucleotide biosynthesis, such as *trpB*, and *leu*, *pur*, and *pyr* operon genes were repressed even 5 h after cold shock. All of these showed further down-regulation in the quadruple-deletion strain.

Cold shock induction of genes encoding heat shock proteins. Protein misfolding was previously not considered a major problem upon cold shock. But increasing numbers of recent reports of a heat shock protein being induced by cold shock even in higher systems suggest that proper folding of proteins as well as refolding of cold-damaged proteins is important after cold shock. However, in most of these cases the heat shock induction of proteins is after prolonged incubation at low temperature (10, 28). On the other hand, in the present study, a number of genes encoding heat shock-inducible proteins and molecular chaperones such as *htpG*, *mopA*, *mopB*, and *ppiA* (encoding HtpG, GroEL, GroES, and peptidyl-prolyl-*cis-trans*-isomerase, respectively) showed transient induction immediately following cold shock. ClpB, which is both heat and cold shock-inducible in *Synechococcus* sp. strain PCC 7942 (28) was induced 10-fold and maintained at this level even at 5 h after temperature downshift (Table 3). *ppiA*, encoding peptidyl-prolyl-*cis-trans*-isomerase, is also reported from *Bacillus*, is involved in accelerating proline-limited steps in protein folding, and is important in helping protein folding at low temperatures (7, 8). Trigger factor encoded by *tig* is another interesting chaperone, which is moderately induced 2 to 3 h after cold shock (15). It is not included in Table 1, as it does not fulfill the criteria of the required *n*-fold increase; however, we did find

moderate (1.7-fold) induction of this gene. Previously, it was also shown that when *E. coli* is grown at 16°C, GroEL expression is reduced (15); however, in that study the cells were grown to an OD_{600} of 0.5, and then the protein expression was checked by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. In the present study, the immediate effect of cold shock is being analyzed (1 h). Both *mopA* and *mopB* were induced 9.3- and 8-fold after 1 h and then reduced to a basal level of 1.3- and 0.9-fold, respectively, after 5 h. Thus, our data are consistent with the low level of GroEL observed by these authors after cells reached the OD_{600} of 0.5 at 16°C. Note that the OD_{600} increased by 0.4 at 5 h after cold shock in the present study. This suggests that the GroELS system is transiently induced immediately after the temperature downshift, along with the induction of CspA homologues, and is then reduced to a basal level. It should be mentioned that GroEL is induced in *E. coli* at 37°C by the overexpression of CspC and CspE, although this induction is lesser than its heat shock induction (22). On the same note, the present study showed that deletion of four CspA homologues leads to repression of cold shock induction of *mopA* and *mopB* (Table 1). This suggests a possibility that cold shock induction of GroEL may be linked to the higher levels of CspA homologues.

Comparison of cold shock response of *E. coli* and *B. subtilis*. DNA microarray analysis of the cold shock response of *B. subtilis* has been carried out by two groups (2, 13). Our study showed that there are a number of common genes such as *leuBCD* (amino acid biosynthesis) and *purBCDEFHKLMN* (purine biosynthesis) that are affected by cold shock in *E. coli* and *Bacillus* spp. Other such examples include *topA* (DNA topoisomerase I), *gltB*, *arg*, and *aro* genes (amino acid biosynthesis) (Table 2). There are certain genes that are not included in the tables, as their ratios do not fulfill the criteria of the required *n*-fold difference; however, these are worth mentioning as they are affected by cold shock in *Bacillus* spp. The genes and the respective *n*-fold differences are as follows: (i) amino acid biosynthesis, *aroF* and *aroH* (0.5 and 0.6, respectively), *metC* (0.7), and *serC* (0.6); (ii) tRNA synthetases, *aspS* (0.6), *hisS* (0.5), and *thrS* (0.75); (iii) NAD biosynthesis, *nifS* (0.7) and *nadC* (0.7); (iv) ATP synthase, *atpA*, *atpB*, *atpE*, *atpF*, *atpH*, and *atpI* (approximately 0.5); (vi) pyrimidine biosynthesis, *pyrC* (0.75); (vii) citric acid cycle, *sdhC* (0.8); and (viii) metabolism, *bioA* and *bioD* (0.5 to 0.6) and *ptb* (2). However, there were also differences between *E. coli* and *Bacillus* cold shock response. For example, in the case of *Bacillus* spp., the ribosomal proteins were induced by cold shock, while in *E. coli*, these were either repressed or showed no significant change. One of these two analyses in *Bacillus* showed repression of GroEL 70 min after cold shock (2), while the present study shows induction of the GroELS system in *E. coli* 1 h after cold shock. This suggests that in spite of common basic principles in the cold shock response of *E. coli* and *Bacillus* spp., there are certain distinct differences.

CspA homologues are needed at acclimation phase. At low temperature, the secondary structures of RNA stabilize, which should slow down (i) transcription elongation and (ii) ribosomal movement on RNA and thus translation. The Csps are transiently and dramatically induced in the acclimation phase upon cold shock. These presumably act as RNA chaperones (1, 9, 11, 23, 25) by destabilizing the secondary structures in RNA and thus facilitating transcription and translation. Increased levels of CspA homologues after cold shock may be important for compensating for higher stability of secondary structures in RNA at low temperatures (11). The RNA chaperone effect of CspA homologues is apparent in the present microarray analysis, as cold shock induction of a number of diverse genes was repressed by deletion of four *csp* genes. These may be the genes that need help to transcribe and translate efficiently at low temperature, possibly due to stabilization of secondary structures in their mRNAs, and the high level of Csps ensures their effective production. It is noteworthy that, except for the flagellar operon, deletion of four *csp* genes mainly affected genes that are transiently induced during acclimation phase. This emphasizes the need for the RNA chaperones immediately upon cold shock, and once the cells are acclimated to cold, their presence is no longer required. This is supported by the observations that cold shock induction of Csps is transient and the quadruple-deletion strain shows a prolonged lag period after cold shock. Further studies on the effect of Csps on the transcription and translation of genes, especially those encoding GroELS, maltose, the ribose operon, and flagellar proteins, should prove to be useful in this aspect.

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