Arch Microbiol (2015) 197:497–501
DOI 10.1007/s00203-015-1085-4

SHORT COMMUNICATION

Cyclic AMP receptor protein (CRP) regulates the expression of *cspA*, *cspB*, *cspG* and *cspI*, members of *cspA* family, in *Escherichia coli*

Sheetal Uppal · Narendra Jawali

Received: 26 September 2014 / Revised: 14 January 2015 / Accepted: 16 January 2015 / Published online: 31 January 2015

Abstract *Escherichia coli* K-12 contains nine paralogs of CspA, CspA–CspI, collectively known as CspA family of cold-shock proteins (CSPs). In spite of the high degree of similarity among themselves, only five (*cspA*, *B*, *E*, *G* and *I*) are induced during cold-stress. In the present study, we show that *cspB*, *cspG* and *cspI*, the members of *cspA* family, known to be induced in response to cold shock, are regulated by cyclic AMP receptor protein (CRP), a global regulator involved in sugar metabolism, during growth at 37 °C as well as at 15 °C, as seen by green fluorescent protein (*gfp*) promoter fusions assays. Interestingly, *cspA* is selectively regulated by CRP during growth at 15 °C but not at 37 °C. The regulation of *cspA*, *cspB*, *cspG* and *cspI* by CRP was found to be through an indirect mechanism as determined by electrophoretic mobility shift assay (EMSA). These results substantiate our earlier study demonstrating a role for CRP during growth at low temperature.

Keywords CRP · coli · csp · Cold · Regulation · Transcription

Escherichia coli K-12 contains nine paralogs of CspA, CspA–CspI, collectively known as CspA family of cold-shock proteins (CSPs). Members of this family are small proteins consisting of a nucleic acid-binding domain called the cold-shock domain (CSD), one of the most evolutionarily conserved domains found in various life forms including bacteria, plants and animals (Wolffe 1994; Graumann and Marahiel 1998). CspA and some of its homologs share nucleic acid melting ability (Jiang et al. 1997; Phadtare et al. 2002) and transcription anti-termination activity (Bae et al. 2000), the two functions important for cold adaptation (Phadtare et al. 2002). In spite of the high degree of similarity among themselves, only five (*cspA*, *B*, *E*, *G* and *I*) are induced during cold shock (Gualerzi et al. 2003; Uppal et al. 2008). *cspC*, involved in regulation of growth (Rath and Jawali 2006), is constitutively expressed at 37 °C, while *cspD* is induced during starvation (Yamanaka and Inouye 1997; Uppal et al. 2014). The transcription from both *cspE* and *cspD* promoters is activated by cyclic AMP (cAMP) receptor protein (CRP), a global metabolic regulator (Uppal et al. 2011, 2014). CRP, also known as catabolite activator protein (CAP), is responsible for regulation of genes involved in various metabolic pathways in *E. coli* (Kolb et al. 1993). The absence of CRP leads to a growth defect at low temperature (Uppal et al. 2014).

In the present study, we show that *cspB*, *cspG* and *cspI*, the members of *cspA* family and induced in response to cold shock (reviewed by Gualerzi et al. 2003), are regulated by CRP. To investigate the role of CRP in regulating the cold-inducible *csps*, p*cspA-gfp*, p*cspB-gfp*, p*cspG-gfp* and p*cspI-gfp*, the *gfp* promoter fusions [Open Biosystems Inc. (Zaslaver et al. 2006)] for *cspA*, *cspB*, *cspG* and *cspI*, respectively, were used. Both M182 [*E. coli K12* Δ (*lacIPOZY*) × *74galK galU strA*] and M182Δ*crp* strains were transformed with the transcriptional fusions. Freshly diluted cultures were grown in 96-well plate without shaking. Both GFP-mut2 fluorescence (excitation: 480 nm and emission: 510 nm) and the absorbance of the culture at 600 nm were measured (OD_{600}) in a multiwell plate reader (Infinite200, Tecan, UK). The specific fluorescence intensity (SFI), defined by fluorescence signal per cell, was calculated for growth at 37 °C (Fig. 1). Since GFP-mut2 variant is highly stable and non-toxic to the cells,

Communicated by Erko Stackebrandt.

S. Uppal (✉) · N. Jawali
Molecular Biology Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400085, India
e-mail: sheetal@barc.gov.in

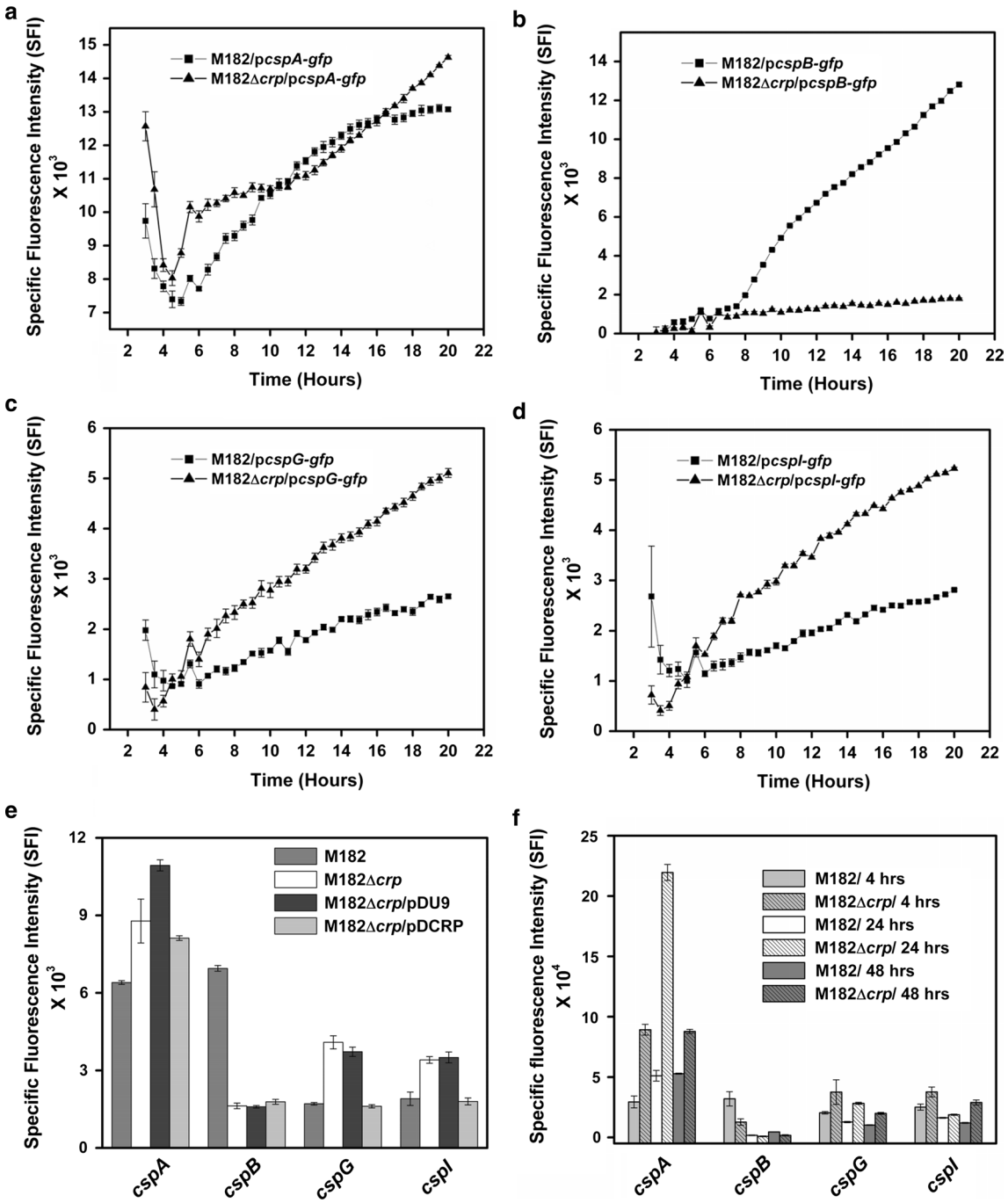

Fig. 1 Plot of specific fluorescence intensity (SFI), expressed in arbitrary units (A.U.), against time for M182 and M182Δ*crp* cells harboring the GFP fusions for the respective genes **a** p*cspA-gfp* for *cspA*, **b** p*cspB-gfp* for *cspB*, **c** p*cspG-gfp* for *cspG* and **d** p*cspI-gfp* for *cspI*. The specific fluorescence intensity is calculated as the ratio of fluorescence and OD_{600}. The upstream region present in the fusion plasmids p*cspA-gfp*, p*cspB-gfp*, p*cspG-gfp* and p*cspI-gfp is* spanning −213 to +214 for *cspA*; −203 to +234 for *cspB*; −205 to +239 for *cspG*; and −292 to +217 for *cspI*, respectively. **e** A *bar diagram* showing SFI levels after 14 h of inoculation in a plate assay for M182, M182Δ*crp*, M182Δ*crp* cells harboring pDU9 (control) and pDCRP (WT CRP) for *cspA*, *cspB*, *cspG* and *cspI*, respectively. The plate assay was carried out essentially as described in Uppal et al. (2011). **f** SFI levels from individual transcriptional GFP fusions (for *cspA*, *cspB*, *cspG* and *cspI*) in M182 and M182Δ*crp* cells after 4, 24 and 48 h of inoculation during continuous growth at 15 °C. The cultures were grown in a microtitre plate at 15 °C with continuous shaking. All the experiments were repeated thrice and the SFI data corresponds to the mean of three independent repeats with *error bars* showing the standard error

the accumulation of the fluorescence per cell (SFI) serves as the reporter of the promoter activity (Zaslaver et al. 2006). For all the genes, the SFI levels showed continuous increase during growth at 37 °C in the wild type cells (Fig. 1a–d). For *cspA*, the SFI levels followed an almost similar pattern in both *crp* knockout cells and the wild type cells indicating no regulation by CRP (Fig. 1a). For *cspB*, the SFI levels were significantly lower (maximum ~7 fold) in *crp* knockout cells compared to the wild type cells (Fig. 1b) suggesting a positive regulation of *cspB* by CRP. In contrast to *cspB*, both *cspG* and *cspI* showed an increase in the SFI level in the knockout cells as compared to the wild type cells (Fig. 1c, d) indicating that both *cspG* (~1.9 fold) and *cspI* (~1.8 fold) are negatively regulated by CRP. These results suggest that CRP up-regulates *cspB* and down-regulates *cspG* and *cspI*. This was further confirmed by complementing M182Δ*crp* cells with pDCRP (West et al. 1993), a multicopy plasmid having *crp* and pDU9, plasmid control, respectively (Fig. 1e). The presence of multicopy *crp* marginally decreased *cspA* expression, while both *cspI* and *cspG* showed a ~1.9 and ~2.3 fold decrease thereby confirming that both *cspG* and *cspI* are down regulated by CRP. In contrast, *cspB* level did not show any change after complementation with multicopy *crp*. It is possible that *cspB* promoter responds differently to the multiple copies of *crp* gene in trans on a plasmid as compared to the single copy of *crp* present on the chromosome.

Though above results confirm that, among the four cold-induced *csps*, only *cspB*, *cspG* and *cspI* are regulated by CRP at 37 °C, the role of CRP in regulating their expression at low temperature still needs to be addressed. For this, the effect of *crp* mutation on their expression during growth at 15 °C was analyzed. Both M182 and M182Δ*crp* strains harboring respective transcriptional fusions (for *cspA*, *B*, *G*, *I*) were grown continuously at 15 °C. The SFI levels were measured at 4, 24 and 48 h after inoculation (Fig. 1f). For *cspB*, *crp* deletion resulted in decreased SFI levels during initial stage of growth only, while *cspG* levels were consistently higher in *crp* deletion strain. For *cspI*, *crp* deletion resulted in higher SFI levels at 4 and 48 h, while the levels at 24 h did not show any difference as compared to the wild type. Interestingly, *crp* deletion caused two to fourfold increase in *cspA* levels at all stages of growth at 15 °C. These results indicate that CRP regulates *cspA*, *cspB*, *csp G* and *cspI* during different stages of growth at low temperature.

The CRP-mediated regulation could be either by direct binding of CRP to the gene promoter [e.g., *cspE* (Uppal et al. 2011) and *cspD* (Uppal et al. 2014)] or indirect, where CRP might regulate a primary regulator of the gene being analyzed. Sequence analysis of the promoter region corresponding to *cspA*, *B*, *G* and *I* revealed putative CRP target sites (Fig. 2a). Interestingly, the putative CRP target sites in *cspA*, *cspG* and *cspI*, the genes negatively regulated by CRP

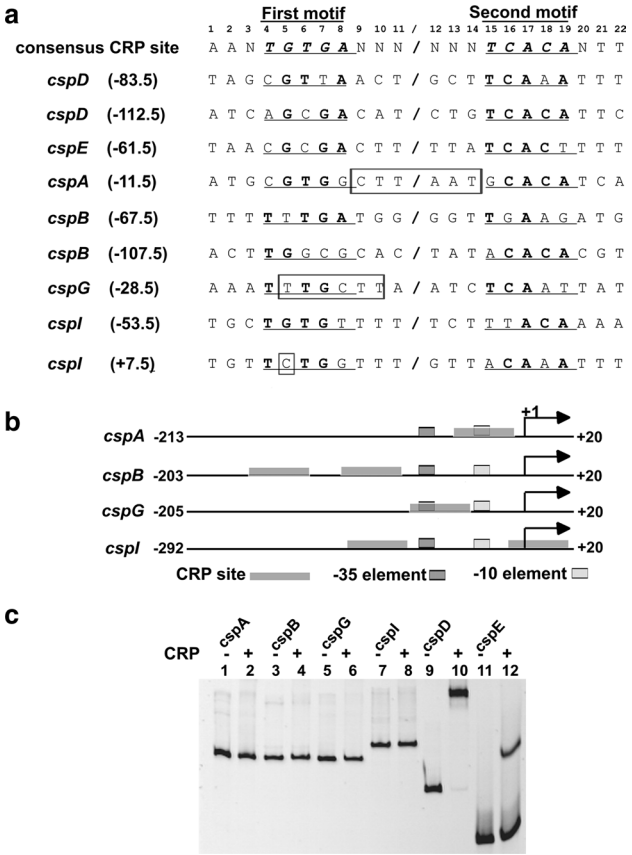

Fig. 2 **a** The 22 b consensus CRP target site sequence and the putative CRP target sites in *cspD*, *cspE*, *cspA*, *cspB*, *cspG* and *cspI* are aligned against each other. CRP target site is a palindromic sequence, where two conserved core motifs, having the five-base-pair sequence TGTGA, are separated by a six-base-pair spacer. The numbering of the nucleotide positions in the target site is essentially same as used by Ebright et al. (1984). The first conserved core motif is from nucleotide positions 4–8 and the second core motif is from 15 to 19. Nucleotide positions 9–14 is termed as spacer region in the CRP target site. The position of the dyad axis of the target sites is mentioned. The residues identical to the consensus are shown in *bold* and the two core motifs are *underlined*. The position of −10 element, −35 element and transcription start site overlapping with the CRP target sites in *cspA* (−11.5), *cspG* (−28.5) and *cspI* (+7.5), respectively, are shown by a *rectangular box*. **b** A *pictorial* representation of the positions of the CRP sites with respect to the gene promoter in the *upstream* region sequence (same as present in *gfp* transcriptional fusions) of *cspA*, *cspB*, *cspG* and *cspI* (figure not to scale). **c** Electrophoretic mobility shift assays (EMSA) probing binding of CRP (~200 nM) to the upstream region of *cspA*, *cspB*, *cspG*, *cspI*, *cspD* and *cspE*, genes using an 8 % polyacrylamide gel

(Fig. 1), overlap with −10 element, −35 element and transcription start site of the gene promoter (Fig. 2b), respectively. In case of *cspB* and *cspI*, two CRP target sites were predicted. In order to find out whether CRP binds to the promoter region of *cspA*, *B*, *G* and *I*, electrophoretic mobility shift assay (EMSA) was carried out essentially as described in Uppal et al. (2011). The DNA fragments spanning the upstream regions (corresponding to the upstream region

Table 1 Primers used in this study

Primer	Sequences (5′–3′)
SU40-E-F	TAAGTAACATCAAAAATAAC
SU43-E-R	AAATGCTGTGTCGGTTACCA
SU80-D-F	TCACATTCCTGTCAATAGCGT
SU81-D-R	AGGTATGAATCAAAAATTTG
SU87-A-F	GAATGGGAATCCAGACGCGT
SU88-A-R	AGGAGTGATGAAGCCGAAGC
SU89-B-F	TGGGGTGATAAGACCCTTGC
SU90-B-R	CCATCAACAGGAGAAATAAA
SU91-G-F	TCTTTACGACCGTCGGAGGG
SU92-G-R	CGTCTTTGCTGCCATCATCAG
SU93-I-F	TGCTGAAATTGCTGACGAAC
SU94-I-R	TCGGCGTGATGAAACCAAAA

present in the respective transcriptional fusions) of these genes were amplified using MG1655 chromosomal DNA as a template. The primers used (Table 1) were as follows: SU87-A-F and SU88-A-R for *cspA* spanning positions −213 to +214, SU89-B-F and SU90-B-R for *cspB* spanning positions −203 to +234; SU91-G-F and SU92-G-R for *cspG* spanning positions −205 to +239; SU93-I-F and SU94-I-R for *cspI* spanning positions −292 to +217; SU82-D-F and SU81-D-R for *cspD* spanning positions −274 to +1; and SU40-E-F and SU43-E-R for *cspE* spanning positions −88 to +12 in the upstream region. These fragments with and without CRP (~200 nM) were electrophoresed on an 8 % polyacrylamide gel in the presence of cAMP (200 μM). As CRP is known to bind to *cspE* (Uppal et al. 2011) and *cspD* (Uppal et al. 2014), these genes were also included in the analysis as positive controls. In line with previous results (Uppal et al. 2011, 2014), CRP showed binding to both *cspD* and *cspE* (Fig. 2c). However, in spite of showing a match to the consensus CRP site (Fig. 2a), the putative target sites in *cspA*, *cspB*, *cspG* and *cspI* did not show binding to CRP. It may be possible that the putative sites in these genes have become degenerate and lost the binding function along the evolutionary process. Alternatively, the putative sites may have very week binding affinity for CRP and may only function in the presence of other hitherto unknown factor(s). Nevertheless, the above results indicate that the regulation of *cspA*, *cspB*, *cspG* and *cspI* by CRP is likely to be through an indirect mechanism. This indirect regulation could be due to a transcriptional regulator whose activity is controlled by CRP or could be via CRP-activated small non-coding RNAs (e.g., CyaR) which are induced as a result of nutritional depletion (DeLay and Gottesman 2009). One cannot also rule out the regulation due to changes in DNA topology as CRP is known to regulate *gyrA* levels in the cell (Gomez-Gomez et al. 1996).

E. coli adapts to low temperature by inducing a cold-shock response which, in addition to many other classes of genes, includes induction of some of the *cspA* family members (*cspA*, *cspB*, *cspE*, *cspG* and *cspI*) (Gualerzi et al. 2003). The RNA chaperone activity and nucleic acid melting ability of Csps help to facilitate translation/transcription/anti-termination by resolving nucleic acid secondary structures at low temperature (Jiang et al. 1997; Bae et al. 2000; Phadtare et al. 2002). In a previous study (Uppal et al. 2011), we have shown that CRP activates *cspE* and the presence of CRP is critical for continuous growth at 15 °C indicating an important role for CRP during growth at low temperature. The present study reinforces and widens this role of CRP by showing that CRP regulates cold-induced *cspA* family members during growth at 37 °C (*cspB*, *cspG*, and *cspI*) as well as at 15 °C (*cspA*, *cspB*, *cspG* and *cspI*). It will be pertinent to mention here that CRP regulates *cspA* expression selectively during growth at low temperature. Similar to this, H-NS-mediated repression of *virF* happens selectively at low temperature, presumably due to altered level of DNA supercoiling, in *Shigella flexneri* (Drlica and Rosenthal 1999). The mechanism of the low temperature specific CRP-mediated regulation of *cspA* is not yet known.

Overall, this study demonstrates that CRP regulates multiple cold-induced *cspA* family members, albeit indirectly, during normal growth and at low temperature growth. A balanced level of these proteins seems to be important for optimal growth at low temperature (Xia et al. 2001). Also, prolonged expression of CSPs is known to adversely affect growth at low temperature (Yamanaka and Inouye 2001). Based on our results (present study; Uppal et al. 2011), we speculate that the growth defect conferred by *crp* deletion (Uppal et al. 2011) might be a result of altered transcription level of *csp* genes. As CRP regulates more than 70 genes encoding transcription regulators (Shimada et al. 2011), it is difficult to identify the factor(s) downstream of CRP mediating the indirect regulation of *csps*. Nonetheless, this study corroborates several earlier reports which have indicated a cross-talk between the cellular metabolic status and cold-shock response (Beaufils et al. 2007; Farewell and Neidhardt 1998; Jones et al. 1992; Van-Bogelen and Neidhardt 1990; Uppal et al. 2011). In addition, global gene expression profiling has indicated that many cold-induced genes may be subject to catabolite repression (Gosset et al. 2004; Phadtare and Inouye 2004). It would be interesting to explore if CRP facilitates a cross-talk between cellular metabolic status and cold-shock response.

Acknowledgments We are extremely grateful to Dr. S. Busby for the kind gift of strains and plasmids.

References

Bae W, Xia B, Inouye M, Severinov K (2000) *Escherichia coli* CspA-family RNA chaperones are transcription antiterminators. Proc Natl Acad Sci USA 97:7784–7789

Beaufils et al (2007) The cold shock response of *Lactobacillus casei:* relation between HPr phosphorylation and resistance to freeze/thaw cycles. J Mol Microbiol Biotechnol 13:65–75

DeLay N, Gottesman S (2009) The Crp-Activated small noncoding regulatory RNA CyaR (RyeE) links nutritional status to group behavior. J Bacteriol 191:461–476

Drlica K, Rosenthal NRP (1999) DNA switches for thermal control of gene expression. Trends Microbiol 7:425–426

Ebright RH, Cossart P, Gicquel-Sanzey B, Beckwith J (1984) Molecular basis of DNA sequence recognition by the catabolite gene activator protein: detailed inferences from three mutations that alter DNA sequence specificity. Proc Natl Acad Sci USA 81:7274–7278

Farewell A, Neidhardt FC (1998) Effect of temperature on in vivo protein synthetic capacity in *Escherichia coli*. J Bacteriol 180:4704–4710

Gomez-Gomez JM, Baquero F, Blazquez J (1996) Cyclic AMP receptor protein positively controls *gyrA* transcription and alters DNA topology after nutritional upshift in *Escherichia coli*. J Bacteriol 178:3331–3334

Gosset G, Zhang Z, Nayyar S, Cuevas WA, Saier MH Jr (2004) Transcriptome analysis of Crp-dependent catabolite control of gene expression in *Escherichia coli*. J Bacteriol 186:3516–3524

Graumann PL, Marahiel MA (1998) A superfamily of proteins that contain the cold-shock domain. Trends Biochem Sci 23:286–290

Gualerzi CO, Giuliodori AM, Pon CL (2003) Transcriptional and post-transcriptional control of cold-shock genes. J Mol Biol 331:527–539

Jiang W, Hou Y, Inouye M (1997) CspA, the major cold-shock protein of *Escherichia coli*, is an RNA chaperone. J Biol Chem 272:196–202

Jones PG, Cashel M, Glaser G, Neidhardt FC (1992) Function of a relaxed-like state following temperature downshifts in *Escherichia coli*. J Bacteriol 174:3903–3914

Kolb A, Busby S, Buc H, Garges S, Adhya S (1993) Transcriptional regulation by cAMP and its receptor protein. Annu Rev Biochem 62:749–795

Phadtare S, Inouye M (2004) Genome-wide transcriptional analysis of the cold shock response in wild-type and cold-sensitive, quadruple-*csp*-deletion strains of *Escherichia coli*. J Bacteriol 186:7007–7014

Phadtare S, Inouye M, Severinov K (2002) The nucleic acid melting activity of *Escherichia coli* CspE is critical for transcription antitermination and cold acclimation of cells. J Biol Chem 277:7239–7245

Rath D, Jawali N (2006) Loss of expression of *cspC*, a cold shock family gene, confers a gain of fitness in *Escherichia coli* K-12 strains. J Bacteriol 188:6780–6785

Shimada T, Fujita N, Yamamoto K, Ishihama A (2011) Novel roles of cAMP receptor protein (CRP) in regulation of transport and metabolism of carbon sources. PLoS One 6(6):e20081. doi:10.1371/journal.pone.0020081

Uppal S, Akkipeddi VS, Jawali N (2008) Posttranscriptional regulation of *cspE* in *Escherichia coli*: involvement of the short 5′-untranslated region. FEMS Microbiol Lett 279:83–91

Uppal S, Maurya SR, Hire R, Jawali N (2011) Cyclic AMP receptor protein (CRP) regulates *cspE*, an early cold-inducible gene, in *Escherichia coli*. J Bacteriol 193:6142–6151

Uppal S, Shetty DM, Jawali N (2014) Cyclic AMP receptor protein regulates *cspD*, a bacterial toxin gene, in *Escherichia coli*. J Bacteriol 196:1569–1577

VanBogelen RA, Neidhardt FC (1990) Ribosomes as sensors of heat and cold shock in *Escherichia coli*. Proc Natl Acad Sci USA 87:5589–5593

West D, Williams R, Rhodius V, Bell A, Sharma N, Zou C, Fujita N, Ishihama A, Busby S (1993) Interactions between the *Escherichia coli* cyclic AMP receptor protein and RNA polymerase at class II promoters. Mol Microbiol 10:789–797

Wolffe AP (1994) Structural and functional properties of the evolutionarily ancient Y-box family of nucleic acid binding proteins. Bioessays 16:245–251

Xia B, Ke H, Inouye M (2001) Acquirement of cold sensitivity by quadruple deletion of the *cspA* family and its suppression by PNPase S1 domain in *Escherichia coli*. Mol Microbiol 40:179–188

Yamanaka K, Inouye M (1997) Growth-phase-dependent expression of *cspD*, encoding a member of the CspA family in *Escherichia coli*. J Bacteriol 179:5126–5130

Yamanaka K, Inouye M (2001) Selective mRNA degradation by polynucleotide phosphorylase in cold shock adaptation in *Escherichia coli*. J Bacteriol 183:2808–2816

Zaslaver A, Bren A, Ronen M et al (2006) A comprehensive library of fluorescent transcriptional reporters for *Escherichia coli*. Nat Methods 3:623–628