Expression of the cold-shock gene *cspB* in *Salmonella typhimurium* occurs below a threshold temperature

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Previous studies have shown that several bacterial species exhibit a multigenic response following temperature downshift (cold shock). Evidence for such a response in *Salmonella typhimurium* is reported, based on the isolation of a range of low-induction-temperature gene fusions containing *Mudlux* insertions. The fusions exhibited different levels of basal light at 30 °C, and were induced at different rates and to different degrees over several hours following a reduction in temperature to 10 °C. Of the *Mudlux* gene fusions isolated, one was found which produced essentially no light when grown at 30 °C but exhibited rapid and high-level induction when the temperature was reduced to 10 °C. The target of this gene fusion (which was named *cspB*) was shown to lie adjacent to the *umuDC* operon and to encode a homologue of the major cold-shock protein of *Escherichia coli*, CspA. Luminescence studies revealed that substantial light production occurred from the *cspB::Mudlux* fusion at or below 22 °C but not at higher temperatures, even following a temperature drop from 30 °C. Moreover, *cspB* mRNA levels were found to mimic this pattern of luminescence, suggesting that *cspB* expression occurs below a defined temperature threshold. The *cspB* mRNA was also found to be very stable at 10 °C but to become highly unstable when the temperature was raised towards the threshold temperature, even in the presence of rifampicin. Existing cellular RNases therefore appear to mediate the decay of *cspB* mRNA at high temperatures, but are incapable of this at low temperatures.

Keywords: bioluminescence, cold shock, CspB, temperature threshold, *Salmonella typhimurium*

INTRODUCTION

Most eubacteria have the capacity to grow over a temperature range of about 40 °C. In the (normal) mid-range of temperatures, the logarithm of growth rate is inversely proportional to the absolute temperature, whilst above and below the normal range, growth rate is less than predicted by extrapolation of the Arrhenius relationship (Neidhardt *et al.*, 1990). Reduction in temperature below the normal range has been shown to result in a selective increase in the synthesis of specific

subsets of proteins in a wide range of bacteria. In several studies, these proteins have been referred to as the cold-shock proteins or cold-acclimation proteins (Jones *et al.*, 1987; Avgay *et al.*, 1992; Whyte & Inniss, 1992; Willmsky *et al.*, 1992).

Cold-shock proteins have been most extensively characterized in *Escherichia coli* and have been shown to include proteins, such as polynucleotide phosphorylase, NusA, translation initiation factors 2 α and 2 β , dihydro-lipoamide acetyltransferase, pyruvate dehydrogenase, RecA, H-NS, GyrA and the DnaJ and DnaK homologues, encoded by the *hsc* operon (Jones *et al.*, 1987, 1992; Lelivelt & Kawula, 1995), an RNA helicase encoded by *csdA* (Jones *et al.*, 1996) and the ribosomal component encoded by *rbfA* (Jones & Inouye, 1996). By implication therefore, a wide range of cellular activities would appear to be affected in this organism by a sudden reduction in growth temperature. Two-dimensional

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electrophoresis and reporter gene studies have shown that three highly related small DNA-binding proteins, named CspA, CspB and CspG, are the major proteins which are synthesized in this organism following cold shock (Jones *et al.*, 1987; Goldstein *et al.*, 1990; Lee *et al.*, 1994; Nakashima *et al.*, 1996). Database searches have revealed that CspA is a member of a highly related family of RNA- or DNA-binding proteins found in eukaryotes, several of which (the Y-box proteins) bind to the core sequence CTGATTGGCCAA (for recent reviews, see Lee *et al.*, 1994; Wolffe, 1994). The promoters of several (but not all) *E. coli* cold-shock genes have also been shown to contain ATTGG sequences in direct or inverted forms (La Teanna *et al.*, 1991; Jones *et al.*, 1992; Qoronfleh *et al.*, 1992).

In the present study, we report the use of a *Mudlux* element (which encodes genes for bioluminescence) for tagging genes of *Salmonella enterica* serovar Typhimurium (designated *Salmonella typhimurium*) which exhibit low-temperature induction. Our data indicate that a range of genes are involved, which exhibit a variety of basal activities at 30 °C and which are differentially expressed following cold shock. Evidence is provided that one of these genes, which we have termed *cspB*, encodes a homologue of the *E. coli* polypeptide, CspA. Our studies indicate that a specific temperature threshold operates for *cspB* expression and this aspect is further explored.

METHODS

Bacterial strains, plasmids and media. Strains and plasmids are described in Table 1. *S. typhimurium* SL1344 and the construction of a *Mudlux* pool in the *galE* derivative,

MPG202, have been described previously (Francis & Gallagher, 1993). Cells were grown overnight at 30 or 37 °C with shaking in LB containing antibiotics (50 µg ml⁻¹) where appropriate. P22HTint4 was used for transduction and for this purpose, overnight cultures of *galE* mutants were grown in medium containing glucose and galactose, each at 0.2% (w/v), to facilitate infection. Bioluminescent samples were diluted from overnight cultures in appropriate volumes to approximately 10⁴ c.f.u. ml⁻¹ and allowed to enter exponential growth for 1 h before further treatment. Samples (10 ml) were removed at suitable time intervals and light emission was measured immediately (in c.p.m.) from individual samples in a Beckman liquid scintillation counter (model LS1701) for 30 s periods. Mud-P22 lysates were prepared as previously described by Benson & Goldman (1992).

Recombinant DNA techniques. Standard DNA manipulation was carried out as described by Sambrook *et al.* (1989). PCR was based on the method of Scharf *et al.* (1986). Initially, samples were denatured for 5 min (95 °C), followed by 35 cycles of 1 min denaturation (95 °C), 30 s annealing (42 °C) and 2 min elongation (72 °C). Oligonucleotides used for amplification of the *E. coli cspA* gene (for Southern blotting) corresponded to residues 617–634 and the complement of residues 900–917 as published (Goldstein *et al.*, 1990), but with substitution of C and G for residues G and C at bases 915 and 910, respectively. Oligonucleotides used for amplification of the *S. typhimurium cspB* gene for use as a probe in Northern blotting corresponded to residues 498–518 and the complement of residues 753–768 (Fig. 3a), respectively. DNA probes were labelled by random priming as described by Feinberg & Vogelstein (1983, 1984). DNA (both strands) was sequenced with Sequenase (USB) using the dideoxy chain-termination method. The GCG package version 7 (Genetics Computer Group, Wisconsin, MA, USA) was used for sequence analysis.

Southern and Northern blotting were carried out on Hybond-N membranes (Amersham) as described by Maniatis *et al.*

Table 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant characteristics*	Source/reference
Strains		
<i>E. coli</i>		
HB101	F ⁻ <i>hdsS20</i> (x _B ⁻ m _B ⁻) <i>supE44</i> <i>recA13</i> <i>ara-14</i> <i>proA2</i> <i>rpsL20</i> (Str ^R) <i>xyl-5</i> <i>mlt-5</i> <i>supE44</i> λ ⁻	Maniatis <i>et al.</i> (1982)
<i>S. typhimurium</i> †		
SL1344	<i>his</i> ; virulent mouse pathogen	Hosieth & Stocker (1981)
MPG201	<i>galE503 bio-561</i> /MuP1 <i>Mudlux</i> ; used to transduce <i>Mudlux</i> into MPG202; SL1344 derivative	Francis & Gallagher (1993)
MPG202	<i>galE503 bio-203::Tn10 his</i> ; SL1344 derivative	Francis & Gallagher (1993)
MPG291–MPG316	<i>Mudlux</i> derivatives of MPG202, produce light at low temperatures	This study
MPG361	<i>cspB::Mudlux</i> derivative of SL1344	This study
TT15250	<i>zea-3666::MudP</i>	Benson & Goldman (1992)
Plasmids		
pKPF1	pBluescript KS derivative containing <i>cspB::Mudlux</i> junction from PCR cloned into <i>Sma</i> I site	This study
pJEC22	pBluescript KS derivative containing <i>cspB</i> on a <i>Sac</i> I fragment of approximately 5 kb	This study
pBluescript	<i>E. coli</i> cloning plasmid; ColE1 Amp ^R <i>lacZα</i>	Short <i>et al.</i> (1988)

* Str^R, Amp^R and Km^R indicate resistance to streptomycin, ampicillin and kanamycin, respectively.

† All *Mudlux* elements are *MudI* (Km^R *lux cts62*).

(1982). RNA was extracted using the hot phenol method (Sarmientos *et al.*, 1983) and aliquots (15 µg) of each sample were separated on a 1.2% denaturing agarose gel containing formaldehyde (Sambrook *et al.*, 1989) and subsequently blotted. Gels were stained with ethidium bromide and rRNA was examined visually for evenness of loading and RNA quality. As an additional control for RNA quality and loading, blots were routinely stripped in a solution composed of 0.1 × SSC, 0.1% (w/v) SDS and 0.2 M Tris/HCl, pH 7.5 (45 °C for 30 min) and reprobed with an end-labelled oligonucleotide (5' CCGGTAGAGTTGCCCTACTCCGGTTT TAG 3') corresponding to part of the *serU* gene of *E. coli* which encodes the tRNA acceptor for serine (EMBL accession number M10746). Primer extension analysis was carried out as described by Ausubel *et al.* (1992). Primers corresponded to bases 580–597 and 561–576 from the non-coding strand (Fig. 3a). [γ -³²P]ATP (Amersham) and T4 polynucleotide kinase (New England Biolabs) were used for end-labelling primers for direct probing or for reverse transcription, and AMV reverse transcriptase (Boehringer Mannheim) was used for mapping transcriptional start sites.

RESULTS

Isolation of *lit*::Mudlux fusions of *S. typhimurium*

In a previous study (Francis & Gallagher, 1993), we reported the construction of a pool of 65000 independent Mudlux fusions in MPG202. This pool of cells was plated at a suitable density and screened for fusions with a low induction temperature (designated *lit*). Colonies were grown overnight at 30 °C and initially screened by eye to identify light-emitting fusions. The plates were then incubated at 10 °C overnight and screened for fusions which selectively produced light at low temperature. This resulted in 49 *lit* fusions. However, upon rescreening, 16 of these fusions were found to produce unacceptably high levels of basal light before incubation at 10 °C and so were eliminated from further analysis. The induction profiles of the remaining *lit* fusions were examined at various time intervals following initial growth in liquid media at 30 °C and subsequent incubation at 10 °C. Representative bioluminescent data are shown for a selection of these fusions (Fig. 1). It can be seen that the fusions varied considerably in their basal level of luminescence at 30 °C, and also in the degree and the rate of light production over the 6 h period following cold shock. This suggested that a number of target genes were involved. Of the fusions examined, strain MPG300 was found to exhibit minimal levels of light at 30 °C but to be the most rapidly and most highly induced fusion at 10 °C. This fusion was transduced into a *galE*⁺ background, MPG361, for further characterization.

The Mudlux element in MPG361 lies within a *cspA* homologue, adjacent to the *umuDC* operon

CspA has been reported to be the most rapidly and highly induced protein in *E. coli* following temperature downshift to 10 °C (Jones *et al.*, 1987; Goldstein *et al.*, 1990). The extremely low level of light (Fig. 1) which was seen at 30 °C, and the time course and intensity of light production from MPG300 following incubation at

10 °C, suggested that the Mudlux element in these cells may have inserted within an *S. typhimurium* equivalent of the *E. coli cspA* gene. We therefore examined whether evidence for linkage between a *cspA* homologue and the Mudlux element in the MPG300 derivative MPG361 could be obtained by PCR. Primers were designed which corresponded to the first eighteen bases of the coding region (from the ATG) of the sense strand of the *E. coli cspA* gene (Goldstein *et al.*, 1990), and to a twenty base region which corresponded to nucleotide residues 83–64 from the terminus of the β -segment of phage Mu (Kahmann & Kamp, 1979). Amplification of chromosomal DNA from MPG361 resulted in a fragment of approximately 150 base pairs which was subsequently blunt-ended with Klenow polymerase and inserted into the *Sma*I site of pBluescript KS (Short *et al.*, 1988), forming plasmid pKPF1. Sequencing of the fragment revealed a *cspA* homologue which was fused 70 bases downstream from the start of the *cspA* primer, to the terminus of the β -segment of phage Mu (data not shown). The predicted sequence of the ORF from amino acid residues 7–23 was found to exhibit 83% identity to the corresponding region of the *E. coli* CspA polypeptide. We have named the gene *cspB_{St}* since a *Salmonella* homologue with 100% nucleic acid identity to *cspA_{Ec}* has already been entered in the public databases (accession number L23115). Database analysis revealed that the sequence corresponded to a partial ORF which had been identified previously and which lay adjacent to the *umuDC* operon of *S. typhimurium* (Smith *et al.*, 1990).

Strain TT15250 is part of the Mud-P22 rapid mapping set (Benson & Goldman, 1992) for *S. typhimurium* and has been used previously to map the *umu* operon (Smith *et al.*, 1990). When induced with mitomycin, this strain produces a lysate which is highly enriched with DNA between 35.9 and 40.5 genetic minutes. Nucleic acid from this lysate was therefore used to confirm the location of *cspB* by Southern blotting, using the PCR-amplified *cspA* gene of *E. coli* as a probe. From Fig. 2, it can be seen that this lysate does indeed carry a *cspA* homologue. [Our experiments have confirmed the presence of a *cspA* gene in *S. typhimurium* which maps to approximately 79 min (unpublished data). However, this gene is not detected in the blot shown in Fig. 2, under the hybridization conditions used, because of the high level of localized enrichment of DNA which occurs using the Mud-P22 system]. A *Sac*I fragment of approximately 5 kb was subsequently cloned from this lysate into pBluescript KS (forming pJEC22) and the complete sequence of the *cspB* gene was obtained (Fig. 3a). The gene encoded an ORF of 70 amino acids with 64%, 67% and 80% identity to the CspA, CspB and CspG proteins of *E. coli*, respectively. Sequence analysis also revealed that the sequence reported previously to lie between *umuC* and *cspB* (Smith *et al.*, 1990) contained a contiguous duplication (bases 59–151; Fig. 3a) which was not present in our clone. PCR analysis using flanking oligonucleotides has confirmed that only a single copy of this sequence is present on the *S. typhimurium* chromo-

Fig. 1. Light production from *lit::Mudlux* fusions following temperature reduction. Cultures (approx. 10^4 c.f.u. ml $^{-1}$; approx. 100 ml) were incubated for 1 h at 30 °C and samples (10 ml) were taken and briefly monitored for bioluminescence (shown as c.p.m.) in a liquid scintillation counter (time 0). Each of the cultures was then placed at 10 °C in a static water bath. Samples (10 ml) were taken at intervals over a 6 h period and bioluminescence was measured immediately. Off-scale values display infinity symbols (∞). Strains are labelled as follows: MPG295 ($\square$), MPG296 ($\square$ with dots), MPG300 ($\square$ with cross-hatch), MPG302 ($\square$ with horizontal lines), MPG316 ($\square$ with vertical lines), MPG291 ($\square$ with diagonal lines), MPG292 ($\square$ with diagonal lines) and MPG 279 ($\blacksquare$), and values reflect representative data from repeat experiments.

Fig. 2. Identification of a *cspA* homologue in *S. typhimurium*. A Mud-P22 lysate was prepared from TT15250. The nucleic acid was isolated and restricted with *Pst*I, *Pst*I/*Sal*I, *Pst*I/*Eco*RI, *Kpn*I or *Bam*HI/*Eco*RV (lanes 1–5, respectively) and Southern-blotted. The existence of a homologue was determined using the PCR-amplified *cspA* gene of *E. coli* as a probe. The positions of λ *Hind*III markers are indicated on the right.

some (data not shown). Primer extension studies using RNA isolated from cells at 10 °C revealed a single transcriptional start point for *cspB* which lies 145 bases upstream of the translational initiation codon (Fig. 3b).

Bioluminescence from MPG361 occurs below a threshold temperature

To explore the pattern of light production from MPG361 more fully, we examined whether a large temperature drop was necessary for *cspB* expression. Previous studies in *E. coli* have emphasized that expression of CspA is activated by a temperature downshift, usually of 13 °C or more (Jones & Inouye, 1994;

Etchegaray *et al.*, 1996). Exponential cultures of MPG361 were therefore grown continuously from low cell density at fixed temperatures or were initially grown at the fixed temperature for 1 h and were then subjected to a temperature reduction. At suitable time intervals, samples were taken and directly examined for light production in a liquid scintillation counter. The results shown in Fig. 4 are for samples which were initially grown at 30 or 24 °C, with or without a further temperature downshift to 24 or 22 °C. It can be seen that substantial light induction occurred from MPG361 cells whenever they were shifted to 22 °C, independently of the initial starting temperature, and that light production remained high over the time period examined. In contrast, only low light levels could be detected above this temperature, even after a temperature reduction of 30 °C to 24 °C. This suggests that a large temperature downshift is not a necessary requirement for induction of *cspB* in *S. typhimurium*, but that substantial gene expression occurs at or below a temperature threshold of approximately 22 °C.

The threshold temperature for light expression correlates with *cspB* mRNA levels and involves differential mRNA stability

Analysis of the sequence of the insert in pKPF1 revealed that an additional T residue lies between residues G₂₉ and T₃₀ of the published sequence of the β -end of the phage Mu sequence (Kahmann & Kamp, 1979) and that a continuous ORF encoding a polypeptide of 71 amino acids had formed between the *cspB* gene and the *Mudlux* element. It therefore remained possible that light production from MPG361 at or below 22 °C (Fig. 4) reflected control at the transcriptional or translational level. To explore the situation further, we examined the levels of *cspB* mRNA following downshifts from 30 °C to either 24 °C, 22 °C or 10 °C and subsequent in-

(a)

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1  AGAAATGCTTTGCGCTCGGTACACCACAAGATACTCTGATCTACTGCGTGTAAAGTAACT
   E M L S P R Y T T R Y S D L L R V K *
   UmuC
61  tgtggatcaatgcctgagatgggtgccaatcatcccggttctcttaacgggttttggtc
121 gcacaagatcacaggaacctctcagatgagGCGCATGTATCTGGTTTACGACATCAGA
181 AAATGTGCGCGTTTATTGCCCCGCGAGCGTTGTGAGACGTCACCTATTTACGCCAGGTT
241 TCAGCGTAGCGACAGGCATGAATAAAAGAGTATGGCAATCAGCGTGATAATGCTAAAA

301  AACAAATTAATATTTTTTAAACAAACTAAAGCTTGCTATGTTTCAGTTAACCATGCGTTAA
   UP element          -35          -10
361  TGGTTGTGCGGTTTGATACAACTTATCTGAAGTAGTGATTGTAATATTTCTCATCATTT
   (+1)
421  GTTCCTCTTGAGATCTCCTTTAGGTTTTTTTCTCTCTGATAATTTCTTCAGGCCATTTT

481  CCGCAAGGCTCATTGCAAGGTAAATATTTATGACGACGAAAATCACTGGTTTAGTAA
           M T T K I T G L V K
           CspB
541  AATGGTTTAAACCTGAAAAGGGCTTTGGTTTCATTACGCCCTAAAGATGGCAGCAAAGATG
           W F N P E K G F G F I T P K D G S K D V

601  TGTGTGTGTCATTTTTCAGCCATTCAAAGTAATGAATCCGCACTCTGAATGAAAATCAGG
           F V H F S A I Q S N E F R T L N E N Q E

661  AAGTGGAGTTTTCAGTAGAGCAGGGACCAAAAGTCCATCAGCGGTCAACGTTGTGGCGC
           V E F S V E Q G P K G P S A V N V V A L

721  TTTAAGGCAACTGATATTACTAATAAAATTCACCTTCGGGTGTCCATGTTGCCATGGTTCA
           *
781  CAATACAGAACATCGACATTCGATGTTACTGAGCAAAA

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Fig. 3. (a) Nucleotide sequence of the *cspB* gene of *S. typhimurium*. The sequence is shown from the 3' end of *umuC* (Smith *et al.*, 1990) to beyond the translational stop codon of *cspB*. Amino acids are shown by the standard one letter abbreviation below the nucleotide sequence. Termination codons are indicated by asterisks. The proposed -35 and extended -10 sequences are underlined, as are the putative Shine-Dalgarno box and translational initiation codon of *cspB*. The transcriptional start site (+1) and the region which is duplicated in the previous report on the *umu* operon (Smith *et al.*, 1990) is shown in lower case letters. The putative UP element is italicized. (b) Identification of the transcriptional start site of *cspB*. Primer extension analysis was carried out on RNA from SL1344 following incubation at 10°C for 1 h. Primers 1 and 2 corresponded to bases 580–597 and 561–576 of the non-coding strand of *cspB* (Fig. 3a) and were used both for primer extension (lanes 1, 6, 7 and 12) and sequencing of pJEC22 (lanes 2–5 and 8–11, respectively). The sequence is shown as GATC (lanes 2–5 and 8–11, respectively) and the transcriptional start site is indicated (arrows).

Fig. 4. Light induction from MPG361 occurs at a specific temperature threshold. An overnight culture of MPG361 was diluted and incubated for 1 h at 30°C or 24°C . When the cultures (approx. 250 ml) reached approximately 10^4 c.f.u. ml^{-1} they were divided (time 0) and the 30°C samples were incubated at either 24°C ($\blacktriangle$) or 22°C ($\blacksquare$) in a water bath whilst the 24°C samples were maintained at 24°C ($\triangle$) or were incubated at 22°C ($\bullet$). Samples (10 ml) were taken at appropriate intervals and bioluminescence was detected immediately in a liquid scintillation counter.

cubation for 1 h. The results (Fig. 5a) show that the level of *cspB* mRNA correlates with the pattern of light production observed in Fig. 4. No *cspB* band was observed following a temperature downshift from 30°C to 24°C , yet a clear band was observed from a temperature downshift to 22°C . Moreover, temperature downshift to 10°C was observed to result in a further increase in *cspB* mRNA when compared with the 22°C sample.

The above studies (Figs 4, 5) showed that light production from MPG361 occurred below a defined temperature of approximately 22°C and that this correlated with the levels of *cspB* mRNA observed above and below the temperature threshold. Nevertheless, the basis for how such a temperature-regulated switch may operate was still unclear. Previous *in vivo* footprinting studies in *E. coli* (Tanabe *et al.*, 1992) provided evidence for selective protection of the *cspA* promoter region at low temperature, and also for a cold-shock-specific protein which binds adjacent to the protected region. More recent studies (Brandi *et al.*, 1996; Goldenberg *et al.*, 1996; Fang *et al.*, 1997) have suggested that differences in mRNA stability at 37°C and 10°C are a key component in regulating *cspA* expression in *E. coli*. Our data suggested that temperature-dependent differences in mRNA stability also play a role in the mechanism of *cspB* expression in *S. typhimurium*. We therefore decided to examine the relationship between *cspB* mRNA stability and temperature more specifically and to explore whether such differences in stability are dependent on *de novo* gene expression. To address these

Fig. 5. *cspB* mRNA is produced below a specific temperature threshold and is destabilized at higher temperatures. The levels and stability of *cspB* mRNA were examined at different temperatures. Exponential cultures of SL1344 cells were initially incubated at 30 °C. The culture was then divided and cooled to 24, 22 or 10 °C in water baths (a, lanes 1, 2 and 3, respectively). RNA was isolated and then probed by Northern blotting. The stability of *cspB* mRNA was also examined following temperature upshift. For this purpose, SL1344 cells were initially cold shocked for 1 h at 10 °C. Rifampicin was then added to a concentration of 200 µg ml⁻¹ and after 20 min (time 0) the cultures were incubated at 10 °C (b) or 24 °C (c). Samples were taken at intervals and analysed by Northern blotting. An aliquot of mRNA from SL1344 cells which had been incubated at 10 °C for 1 h was included as an internal control for autoradiography (labelled as C). The *S. typhimurium cspB* gene was used as a probe in each case and the position of the *cspB* mRNA is indicated (arrows).

issues, exponential cultures of SL1344 were therefore cold-shocked for 60 min at 10 °C to allow accumulation of *cspB* mRNA. Rifampicin was then added and the cultures were subsequently maintained at 10 °C or were incubated at 24 °C. Under these conditions (Fig. 5b, c), it can be seen that the *cspB* mRNA is highly stable at 10 °C but becomes unstable when the incubation temperature is raised to 24 °C.

DISCUSSION

A range of studies using two-dimensional gel electrophoresis have shown the widespread induction of cold-shock proteins in eubacteria following temperature

reduction. We have utilized an alternative approach which exploits the use of a light-based reporter system in *S. typhimurium* to tag genes which exhibit induction at low temperature. The patterns of gene expression suggest that the response is multigenic and that the majority of the genes exhibit some degree of expression independently of cold shock. These findings are in agreement with the studies of Qoronfleh *et al.* (1992) who reported the use of Tn5::lacZ to tag cold-shock genes in *E. coli*.

The nature of the majority of tagged genetic loci of *S. typhimurium* isolated in this study remains largely unknown at present. However, the target of the Mudlux insertion in MPG361 revealed that it encodes a homologue of the CspA family of proteins, three of which, CspA, CspB and CspG, have been shown to be induced in *E. coli* following cold shock. The *cspB_{St}* gene was found to be located downstream of the *umu* operon between 35.9 and 40 min and to be transcribed in a clockwise direction. The location of the *E. coli cspB* gene at approximately 35 min would appear coincidental since this gene lies within a region of inversion between the genomes of these two organisms and no equivalent gene lies adjacent to the *umu* locus of *E. coli*.

The *cspB_{St}* gene was found to be expressed from a single transcriptional start site 145 bp upstream of the translation initiation codon. Assignment of -35 and -10 regions revealed that the -10 box was identical to that of *cspB_{Ec}* and *cspG_{Ec}*. Closer inspection (Fig. 6) revealed the presence of a TGN motif 5' to the -10 sequence in *cspB_{St}* and also, in the corresponding regions of the *E. coli cspA*, *cspB* and *cspG* genes, the *Bacillus subtilis cspB* gene and the *Lactobacillus plantarum cspL* gene (although not in the *cspP* gene of this organism). Such sequences constitute an 'extended -10 promoter' and are characteristic of promoters in which the -35 box is dispensable (Kumar *et al.*, 1993). Moreover, an extensive AT-rich region was also identified at around positions -40 to -60 of *cspB_{St}*, suggesting the presence of an UP element. Although less evident, AT-rich sequences were also identifiable in the corresponding regions of the above *E. coli*, *B. subtilis* and *L. plantarum* genes. Studies on the *E. coli rrnB* gene have reported that UP elements may enhance gene expression by 30-fold (Ross *et al.*, 1993).

Previous studies on *E. coli* reported rapid and high-level expression of *cspA* mRNA following a temperature downshift from 37 °C (Goldstein *et al.*, 1990; Lee *et al.*, 1994), with a more substantial response occurring after a large downshift to temperatures such as 10 °C (Jones *et al.*, 1992; Etchegaray *et al.*, 1996). Moreover, recent studies (Brandi *et al.*, 1996; Goldenberg *et al.*, 1996) have reported that the *E. coli cspA* mRNA is differentially stabilized at temperatures of 10–15 °C compared to 37 °C. In contrast, mRNA from the *E. coli cspB* gene was found to be absent at higher temperatures but to accumulate below a threshold of approximately 20 °C. In our studies, we report the use of a luminescent reporter system to monitor expression of the *cspB* gene of *S. typhimurium*. We have found that a downshift

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