Growth-phase-dependent transcriptional regulation of the *pcm* and *surE* genes required for stationary-phase survival of *Escherichia coli*

Chuan Li,¹ Pei-Ying Wu² and Mingli Hsieh¹

Author for correspondence: Chuan Li. Tel: +886 4 3896190 ext 50617. Fax: +886 4 3892412.
e-mail: cli@mercury.csmc.edu.tw

Institute of Medicine¹ and
School of Medical
Technology², Chung-Shan
Medical and Dental
College, 110, Section 1,
Chen-Kuo North Road,
Taichung, Taiwan

Two neighbouring genes, *surE* and *pcm*, at 59 min on the *Escherichia coli* chromosome are both required for stationary-phase survival. Operon fusions of the putative promoter regions in front of *surE* (P2) or *pcm* (P3) with the *lacZ* reporter gene were constructed to study the transcriptional regulation of *pcm* and *surE*. Both promoter regions were able to activate β -galactosidase activity in a growth-phase-dependent way in either rich or minimal medium. Induction from both promoters reached the highest level in late stationary phase and was independent of the *rpoS/katF* gene. Spent medium from early as well as late stationary-phase cultures could induce the expression of either promoter even after dialysis or boiling. A high cell density could induce the promoters more rapidly but not to a greater extent. It is proposed that the induction might be correlated with the decline in growth rate of the cells. The induction patterns of either P2 or P3 were very similar. *pcm* can thus be transcribed from both the P2 and P3 promoters that are regulated in similar ways.

Keywords: operon fusion, stationary-phase survival, protein carboxyl methyltransferase

INTRODUCTION

A protein-L-isoaspartyl O-methyltransferase (EC 2.1.1.77) can specifically catalyse the transfer of the methyl groups from S-adenosylmethionine to atypical L-isoaspartyl but not normal L-aspartyl residues in proteins (Clarke, 1985). The wide distribution of the activity of this methyltransferase in the living world from eubacteria (Fu *et al.*, 1991; Li & Clarke, 1992a) to fungi (Johnson *et al.*, 1991), worms (Kagan & Clarke, 1995), plants (Mudgett & Clarke, 1993) and mammals (Ingrosso *et al.*, 1989; O'Connor & Clarke, 1985), and the conservation of the amino acid sequence of the enzyme between species (e.g. 31% identity between human and *Escherichia coli*), indicate a house-keeping role of the enzyme (Fu *et al.*, 1991; Ingrosso *et al.*, 1989). Proteins or peptides with abnormal aspartyl residues derived from spontaneous protein degradation (Harding *et al.*, 1989; Stadtman, 1988) are less reactive (Brennan *et al.*, 1994; Johnson *et al.*, 1987). Since methylation of the L-isoaspartyl residues facilitates the conversion of these residues to the normal form, it has been proposed that the methyltransferase might help to repair damaged

proteins that can accumulate with time (Johnson *et al.*, 1987; McFadden & Clarke, 1982, 1987). *E. coli* mutant strains without the *pcm* gene (encoding protein carboxyl methyltransferase, Pcm) exhibit reduced stationary-phase survival and heat resistance (Li & Clarke, 1992b). These phenotypes are consistent with the hypothesis that the enzyme is involved in the metabolism of L-isoaspartyl-containing proteins that can accumulate.

pcm is located at 59 min on the *E. coli* chromosome (Fu *et al.*, 1991). Another gene, *surE*, that is also involved in the stationary-phase survival of *E. coli* is directly upstream of *pcm* (Li *et al.*, 1994). Survival of Gram-negative bacteria in stationary-phase or nutrient-limited cultures that more closely resemble the conditions in the natural habitats of these organisms has been intensively studied (Kolter *et al.*, 1993; Matin *et al.*, 1989; Roszak & Colwell, 1987). An alternative σ factor, σ^S , encoded by the *rpoS/katF* gene plays a central regulatory role in stationary-phase survival or survival under other stress conditions such as osmotic pressure (Hengge-Aronis, 1993; Lange & Hengge-Aronis, 1991; Loewen & Hengge-Aronis, 1994). The *rpoS* gene product is a transcription factor essential for the induction of some stationary-phase survival genes during the transition from exponential- to stationary-phase growth (Loewen

Abbreviation: Pcm, protein carboxyl methyltransferase.

Table 1. Bacterial strains, plasmids and phages

Strain/plasmid/phage	Description	Source/reference
<i>E. coli</i>		
MC4100	F ⁻ Δ(<i>argF-lac</i>)U169 <i>araD139 rpsL150 ptsF25 fibB5301 rbsR deoC relA1</i>	Lange & Hengge-Aronis (1991)
RH90	MC4100 <i>rpoS</i> 359::Tn10	Lange & Hengge-Aronis (1991)
CL415-2	MC4100(ΔCL415-2)	This study
CL415-3	MC4100(ΔCL415-3)	This study
CL415-2R	RH90(ΔCL415-2)	This study
CL415-3R	RH90(ΔCL415-3)	This study
Plasmids		
pRS415	Operon fusion vector	Simons <i>et al.</i> (1987)
pHJK5-2	<i>EcoRI</i> – <i>Bam</i> HI <i>surEp</i> (P2) fragment in pRS415	This study
pHJK5-3	<i>EcoRI</i> – <i>Bam</i> HI <i>pcmp</i> (P3) fragment in pRS415	This study
pCL1	Re-ligation of a 7.5 kb <i>EcoRI</i> – <i>Sna</i> BI fragment from pMMkatF1	Li & Clarke (1992b)
pCL2	4.5 kb <i>Bam</i> HI– <i>Bsp</i> HI fragment from pMMkatF1 inserted in the <i>Bam</i> HI– <i>Sma</i> I site of pGEM-7Zf+	Li <i>et al.</i> (1994)
Phages		
λRS45	Operon fusion phage vector	Simons <i>et al.</i> (1987)
λCL415-2	λRS45 with ϕ (<i>surEp-lacZ</i>) operon fusion	This study
λCL415-3	λRS45 with ϕ (<i>pcmp-lacZ</i>) operon fusion	This study

& Hengge-Aronis, 1994; Weichart *et al.*, 1993). Whether *pcm* is regulated by *rpoS* is not clear, although it is interesting that *pcm* is only separated by one gene (*nlpD*) from *rpoS* on the *E. coli* chromosome (Ichikawa *et al.*, 1994) and this region appears to be important for stationary-phase survival.

It has been shown that the activity of Pcm can be induced 1.7-fold on transition from exponential-stationary-phase growth (Li & Clarke, 1992b). How the expression of *pcm* is regulated by the growth state and/or environmental factors is not clear. Reasonable promoter segments immediately upstream of *pcm* cannot be identified by comparison with consensus promoter sequences. A putative promoter (designated P2; Li *et al.*, 1994) that is about 1.2 kb upstream of *pcm* was found to be the closest to *pcm* with the best match to consensus *E. coli* RNA polymerase recognition sequences. Primer extension analysis of the 5' terminus revealed a G residue as a single start site 6 bases downstream of the proposed –10 region of the putative P2 promoter (Li *et al.*, 1994). Expression of *surE* immediately downstream of this promoter has been shown by coupled *in vitro* transcription/translation (Li *et al.*, 1994). Since *surE* is immediately upstream of *pcm* and both cistrons overlap by 4 bp, it is very likely that they are in the same operon and that this promoter region is responsible for the transcription of both *surE* and *pcm* (Li *et al.*, 1994). RNA species containing elements of both the *surE* and *pcm* genes have been revealed by reverse transcriptase (RT)-PCR experiments, supporting the existence of the *surE*–*pcm* operon (Li *et al.*, 1994). Nevertheless, a *surE* mutant strain, CL2010, with a kanamycin resistance cassette inserted at a *Bst*EII site in the middle of *surE* in the opposite

direction to *pcm*, expressed 18% Pcm activity compared to that of wild-type cells. Thus, expression of *pcm* in CL2010 is unlikely to be under the control of P2 (Li & Clarke, 1992b). Another promoter (designated P3) located directly in front of *pcm* might be responsible for Pcm activity even though no good promoter can be identified in this region by sequence comparison. In this investigation we constructed operon fusions of both putative promoter regions in front of *surE* and *pcm* (see Fig. 1) with the *lacZ* gene to study the regulation of the expression of both genes at the transcription level.

METHODS

Bacterial strains, plasmids, phages and growth conditions.

The genotypes of the *E. coli* strains, plasmids and bacteriophages utilized in this study are listed in Table 1. Bacteria were grown with aeration in glass tubes or flasks at 37 °C unless otherwise indicated. LB (Difco) or M9 medium (with 0.2% glucose as carbon source) were prepared as described by Miller (1992). When required, ampicillin and X-Gal were added to the medium at concentrations of 100 and 40 µg ml⁻¹, respectively.

Construction of *lacZ* operon fusion plasmids. Using PCR, we prepared segments of putative promoter DNA [P2 (*surEp*) in front of *surE*, and P3 (*pcmp*), the possible promoter region in front of *pcm*; Fig. 1] flanked by 5' *Eco*RI and 3' *Bam*HI sites so that they could be directly inserted into the operon fusion vector pRS415 (Table 1, Simons *et al.*, 1987) in front of the *lacZ* gene. The primers used for PCR were P2L (5' GTACCGAATcCTTCGGTGCCCAACG, corresponding to nt 1258–1283 in Li *et al.*, 1994), P2R (5' CGCTGgGaTCCCATTCGCCACTGCCTG, complementary to nt 1571–1597 in Li *et al.*, 1994), P3L (5' GTCCGGAATTcACGCCGGCCGAATC, corresponding to nt 2088–2113 in Li *et al.*, 1994) and P3R (5' GAACCGGatCCAATTTCCAGCAC-

Fig. 1. Illustration of the position of the P2 and P3 regions used in the *lacZ* fusion constructs. The sequence of the identified -35 and -10 regions of P2 (Li *et al.*, 1994) and the potential Shine-Dalgarno ribosomal initiator sequence of *pcm* (Fu *et al.*, 1991) are underlined. The transcriptional start site of *surE* is marked by #. The overlap of *surE* and *pcm* is shown. The valinyl residue marked by * is the N-terminal residue determined by sequence analysis of the purified *E. coli* L-isoadenyl methyltransferase (Fu *et al.*, 1991).

CCG, complementary to nt +232–257 in Fu *et al.*, 1991). Restriction sites for *EcoRI* and *BamHI* are underlined and the bases that have been changed are indicated in lower case letters. For each reaction, 10 nmol primers, 200 ng template DNA (pCL1), 2.5 U *Taq* DNA polymerase (Promega) and 20 nmol each of the four dNTPs were included in a final volume of 100 μ l with 2.5 mM $MgCl_2$ in standard *Taq* polymerase buffer (50 mM KCl, 10 mM Tris/HCl, pH 9.0, 0.1% Triton X-100). The reaction was performed for 10–20 cycles of 30 s at 95 °C (first cycle, 4 min), 30 s at 60 °C and 30 s at 75 °C (final cycle, 3 min). The reaction products were extracted by chloroform, digested with *EcoRI* and *BamHI* and then ligated to pRS415 that had also been digested with *EcoRI* and *BamHI*. Plasmids pHJK5-2 and pHJK5-3, containing the successful P2 and P3 promoter-*lacZ* fusion constructs, respectively, were confirmed by restriction digestion and DNA sequence analysis and were further used to transform a $\Delta lacZ$ strain, MC4100 (Table 1).

Transferring the *lacZ* operon fusions from plasmids to chromosomes. Phage λ RS45 (Table 1) was used to transfect MC4100 cells harbouring plasmid pHJK5-2 or pHJK5-3, according to the protocols of Simons *et al.* (1987), to move the promoter-*lacZ* constructs into the phage. High titre stocks of the resulting recombinant phages, λ CL415-2 or λ CL415-3, were used to transfect *E. coli* MC4100 or a σ^S -deficient strain, RH90 (Lange & Hengge-Aronis, 1991). Successful blue lysogens on X-Gal/LB plates were used for further studies.

β -Galactosidase assay. β -Galactosidase activities were determined with chloroform/SDS-permeabilized cells using ONPG (Sigma) as substrate using the protocol described by Miller (1992). β -Galactosidase activity is expressed in Miller units. Each value was the mean of two independent assays.

RESULTS

Transcription can be initiated by either P2 or P3

Putative promoters P2 (*surEp*) or P3 (*pcmp*) were fused in front of the reporter *lacZ* gene in the multicopy operon fusion vector pRS415 to form operon fusion plasmids pHJK5-2 and pHJK5-3, respectively (Table

1). The strength of these putative promoter regions was determined by measuring β -galactosidase activity in MC4100 ($\Delta lacZ$) cells transformed with the operon fusion plasmids. Both promoters appeared to be good promoters and could be induced both in growing cells and in cells approaching stationary phase (data not shown). However, the results were not consistent when cells entered stationary phase. In view of the fact that the copy number of the plasmids carrying the reporter fusions might vary as cells entered stationary phase and thus interfere with the results, we further transferred the fusions to the *E. coli* chromosome using recombinant phages to eliminate the copy number problem. The resulting lysogens, CL415-2 and CL415-3, contained P2-*lacZ* or P3-*lacZ* fusions, respectively, in an MC4100 background (Table 1).

Increased P2 and P3 activity on transition from exponential growth to stationary phase

Both P2 in front of *surE* and P3 in front of *pcm* could be significantly induced in a growth-phase-dependent way when CL415-2 or CL415-3 cells were grown in rich medium (LB) (Fig. 2a). Expression from either P2 or P3 was low during early exponential phase (about 30–80 Miller units) but was induced about twofold in mid-exponential phase. As shown in Fig. 2(a), the level of expression continued to increase about 15-fold from P2 (about 800–1000 Miller units) and about 12-fold (about 550–700 Miller units) from P3 when the cells reached stationary phase. The level of expression of the P2 operon fusion was consistently slightly higher than that of the P3 operon fusion when cells reached the transition from late exponential to early stationary phase.

Interestingly, when cells were cultured in minimal medium (M9), the maximum induction of activity in stationary-phase cells from P2 was reduced to about 350 Miller units and that from P3 to about 500 Miller units (Fig. 2b). However, the saturated cell density in minimal medium was lower than that in LB. The maximum β -galactosidase activity in cells in minimal medium was the same as in cells in LB at similar OD_{600} . In minimal medium, P3 became a stronger promoter than P2. These results might reflect different requirements of the *pcm* and *surE* gene products under different nutritional conditions.

The *katF/rpoS* gene is not required for the induction of P2 and P3

In prokaryotes specific σ factors play critical roles in the induction of sets of genes required under certain specific growth conditions. A σ factor, σ^S , has been shown to be a central regulator for stationary-phase survival and is required for the expression of more than 20 genes in early stationary phase (Hengge-Aronis, 1993; Loewen & Hengge-Aronis, 1994). Since either P2 or P3 can be induced as cells enter stationary phase, it is possible that they could be controlled by σ^S . We transferred the *lacZ* fusion constructs to an *rpoS* strain, RH90 (Lange & Hengge-Aronis, 1991), and the resulting lysogens were

Fig. 2. Expression from P2 and P3 at different growth stages. CL415-2 or CL415-3 cultures grown at 37 °C in rich medium (a) or minimal medium (b) were inoculated with overnight cultures at a 1:100 dilution. After growth for about 1 h, the cells were further diluted fivefold to assure exponential growth. β -Galactosidase activities ($\square$, CL415-2; $\circ$, CL415-3) are the means of duplicate measurements and similar results were obtained in at least three independent experiments. The growth curves (OD_{600}) of CL415-2 (—) and CL415-3 (---) are shown.

Fig. 3. *rpoS/katF* is not required for the stationary-phase induction of P2 or P3. CL415-2 and its *rpoS* counterpart CL415-2R (a), and CL415-3 and its *rpoS* counterpart CL415-3R (b) were cultured in LB as described in the legend to Fig. 2. β -Galactosidase activities are the means of duplicate measurements and similar results were obtained in two independent experiments. (a) β -Galactosidase activities: $\square$, CL415-2; $\circ$, CL415-2R. Growth curves: —, CL415-2; ---, CL415-2R. (b) β -Galactosidase activities: $\square$, CL415-3; $\circ$, CL415-3R. Growth curves: —, CL415-3; ---, CL415-3R.

designated CL415-2R and CL415-3R (Table 1). We could not detect differences in *lacZ* expression in either construct compared with that in CL415-2 or CL415-3 (Fig. 3). Similar results were obtained for cells grown in minimal medium (data not shown). The results indicate that although σ^s plays an essential role in the expression of some stationary-phase-expressed genes, the stationary-phase induction of *surE* or *pcm* promoters is independent of σ^s .

Effect of spent LB on P2 and P3 induction

katF and *katE* can be induced as cells enter stationary phase and similar induction can be obtained by spent culture supernatant from early stationary-phase cultures (Mulvey *et al.*, 1990). Whether the stationary-phase

induction of P2 or P3 described above is caused by excreted products as cells enter stationary phase was investigated. Spent LB media from early (OD_{600} about 1.3) or late stationary-phase (overnight) cultures were prepared. CL415-2 or CL415-3 cultures in early exponential phase (OD_{600} about 0.3) were split into the spent or fresh LB. Growth and β -galactosidase activity were then determined every 20–40 min. Comparing the spent LB culture with the control culture at the same OD_{600} , an approximately fivefold induction of P2 could be observed (Fig. 4a). An approximately 2.5-fold induction could be observed for P3 in the spent stationary-phase medium (Fig. 4b). Spent medium from late stationary phase had a similar induction effect (data not shown). If the CL415-2 or CL415-3 cells were grown to mid-exponential phase (OD_{600} about 0.5–0.6), the in-

Fig. 4. Effect of spent medium on P2 or P3 induction. Spent LB medium was prepared from an MC4100 culture grown to an OD_{600} of about 1.3 by filtration through 0.22 μ m filters. The medium was either boiled for 10 min or dialysed against SM buffer (20 mM Tris/HCl, pH 7.6, 100 mM NaCl, 1 mM $MgSO_4$, 0.1 g gelatin l^{-1}) as described by Mulvey *et al.* (1990). CL415-2 (a) or CL415-3 cells (b) in exponential phase (OD_{600} about 0.3–0.35) were divided and resuspended in fresh or spent LB. β -Galactosidase activities for cells resuspended in spent LB ($\square$), boiled spent LB ($\triangle$) and dialysed spent LB ($\circ$). β -Galactosidase activities for control cultures (resuspended in fresh LB) of cells resuspended in spent LB (—), boiled spent LB (---) and dialysed spent LB (····).

duction by the spent medium reduced to about twofold for P2 and 1.5-fold for P3 (data not shown). Nevertheless, cell growth appeared to be restricted when the spent medium was used, as the OD_{600} of the culture increased slowly and could only reach about half of that of the control cultures. We either boiled the spent medium or dialysed it against buffer to analyse the active components responsible for the induction. Interestingly, neither treatment could completely reduce the induction effect of the spent medium, although lower maximum induction was obtained (Fig. 4). Consistent with these results was that some metabolites such as ethanol, acetate, lactate, pyruvate and benzoate at 20 mM could

Fig. 5. Effects of the increase in cell density on P2 and P3 induction. CL415-2 cells (a) or CL415-3 cells (b) were harvested in exponential phase (OD_{600} about 0.2–0.25) and resuspended in fresh LB with equal ($\blacksquare$), half ($\square$), quarter ($\square$) or one-sixth ($\square$) volumes of the original culture. β -Galactosidase activities are the means of duplicate measurements and similar results were obtained in two independent experiments. The numbers on each column are the OD_{600} values of that culture.

not induce either P2 or P3 (data not shown). It is thus likely that the induction by spent LB was not due to specific heat-stable small metabolites or heat-labile factors but might result from physiological changes in cells when exposed to the spent medium.

Effect of cell density on P2 and P3 induction

Whether the growth-phase-dependent induction of P2 and P3 was due to the increasing cell density in the culture was also investigated. We pelleted CL415-2 and CL415-3 cells at exponential phase and resuspended

them in fresh LB with equal, half, quarter, or one-sixth volumes of the original culture. We could not detect any immediate induction from the high cell density cultures after 15 min growth of the resuspended culture. The high density cultures achieved a high level of induction of P2 in less time compared with the normal density culture (Fig. 5a). For example, β -galactosidase activity reached 600 Miller units in the 4- or 6-times concentrated cultures after 85 min while it took more than 155 min for the normal cell density culture to reach a similar level of β -galactosidase activity. A similar induction pattern was obtained for P3 with a lower level of induction (Fig. 5b). Nevertheless, the high density cultures showed even lower P2 or P3 induction when compared with normal density cultures at the same OD₆₀₀ values (Fig. 5). Thus, we could not conclude whether the growth-phase-dependent induction of P2 or P3 was due to the increasing cell density.

DISCUSSION

We have demonstrated that not only is the region in front of *surE*, which was previously proposed to be a potential promoter, a good promoter but also the region in front of *pcm* could be a good promoter. Thus, *pcm* encoding a Pcm required for stationary-phase survival either can be expressed from P2 (*surEp*), following *surE* as in an operon, or can be transcribed independently from the P3 (*pcmp*) promoter. Interestingly, the transcription level from both promoters during exponential growth could be induced in a growth-dependent way as cells approached stationary phase. This induction could be observed when cells were grown in either rich or minimal medium.

pcm is upstream of the *katF/rpoS* gene encoding the σ factor, σ^S , separated by only one gene (*nlpD*, Ichikawa *et al.*, 1994). However, the induction of *surEp* and *pcmp* for these two essential stationary-phase genes is independent of the product of the *rpoS/katF* gene that has been shown to be essential for the expression of a few required stationary-phase genes (Hengge-Aronis, 1993; Loewen & Hengge-Aronis, 1994). This result is consistent with the observation that the presence of extra copies of *katF* in the pMMkatF3 transformant did not increase the level of Pcm (Fu *et al.*, 1991). At the same time, the specific activity of Pcm was the same in both the *katF* wild-type strain (MP180) or *katF*-deficient strain (UM122) (C. Li, unpublished data). Other promoters such as *osmC_{pl}* (Gordia & Gutierrez, 1996) and the promoter of the *glgCAP* operon (Hengge-Aronis & Fischer, 1992) have been reported to be growth-phase-regulated but *rpoS*-independent. The exact mechanism of the induction has not been elucidated.

Spent medium from early or late stationary-phase cells can induce either P2 or P3. This induction was observed even after boiling or dialysis of the spent medium. It is thus likely that the induction was due to growth-phase-dependent physiological changes in the cells rather than secondary metabolites or protein factors excreted to the growth medium, or pH changes in the medium. An

increase in cell density can induce β -galactosidase activity more rapidly but cannot induce the activity immediately, nor to a higher extent. We observed that in all of the experiments the induction usually occurred 30–60 min after the decline of the culture growth rate. We propose that the continuous reduction of the culture growth rate from exponential to stationary phase might co-relate with the continuous increase in P2 and P3 induction. The larger the reduction in growth rate, the greater the induction observed. Abrupt growth rate changes when cells were shifted to the spent medium or to a smaller volume of growth medium lead to large growth rate changes and thus induced the promoters. In this hypothesis it is the decline in the growth rate, not the slow growth rate that can induce the promoters. The low maximum induction of P2 and P3 in cells grown in minimal medium might be due to the slow growth and small growth rate changes as cells approach stationary phase under low nutrient conditions. In bacteria, growth rate changes affect protein synthesis greatly (Bremer & Dennis, 1987). It is reasonable that the protein-repair methyltransferase could be induced when protein synthesis is decreased with reduced growth rate.

The observed growth-phase-dependent induction was probably not due to the *relA* genetic background of strain MC4100 used for the *lacZ* operon fusion studies. We moved the fusions to another Δ *lacZ* strain, MC1000, that was *relA*⁺ and obtained similar induction results (C. Li, unpublished data).

The similarity of the induction of P2 and P3 indicates that both promoters are controlled by similar factors. P2 appears to be a typical σ^{70} promoter with homologous –10 and –35 regions. This has been confirmed by primer extension analysis (Li *et al.*, 1994). However, no sequence homology can be identified between P2 and P3. Further analysis of the *cis*-elements and *trans*-acting factors that are responsible for the similar induction of both promoters is important.

The arrangement of the *surE-pcm-nlpD-rpoS* genes important for stationary-phase survival on the 59 min region of the *E. coli* chromosome has been proposed to be conserved in Gram-negative bacteria (Li *et al.*, 1994; Visick & Clarke, 1995). The genomes of a Gram-negative bacterium (*Haemophilus influenzae*, Fleischmann *et al.*, 1995), a Gram-positive bacterium (*Mycoplasma genitalium*, Fraser *et al.*, 1995) and an Archaeon (*Methanococcus jannaschii*, Bult *et al.*, 1996) have been completely sequenced. Examination of the arrangement of the genes in these bacteria has revealed interesting information. No *pcm* homologue can be identified in *M. genitalium*, consistent with our previous survey in that methyltransferase activity is absent in Gram-positive bacteria (Li & Clarke, 1992a). Interestingly, no *pcm* homologue can be found in *H. influenzae* while a good homologue of *surE* can be identified. The hypothetical *surE* product of *H. influenzae* appeared to be 90 aa shorter at the N terminus. The portion corresponding to the N terminus of *E. coli surE* and the P2 region was proposed to be present in a

Fig. 6. Comparison of the organization of the 59 min region on the *E. coli* chromosome and the arrangement of genes in the 746600–752000 nt region on the *H. influenzae* genome. ORFs are shown as boxes and the direction of all ORFs is from left to right. ORFs with sequences conserved between the two bacteria are represented by hatching. ORFs at a similar chromosomal position but which are not homologous are represented by white (*E. coli*) or black (*H. influenzae*). The nucleotide sequence of the hypothetical protein HI701 of *H. influenzae* is highly conserved (about 55% identity) compared to the nucleotide sequence of the intragenic region between *orf0* and *surE* as well as the 5' coding region of *surE* in *E. coli*.

hypothetical protein, HI701, of *H. influenzae* (see Fig. 6). We examined the degree of homology between the *E. coli* P2 segment from this study and the *H. influenzae* sequence and found 58.5% identity. The 5' half of the P3 region was located in the coding region of *surE* and thus showed a high degree of homology between both bacteria. No homologous sequence could be found in *H. influenzae* for the 3' portion of P3 that extends into the *pcm* coding region of *E. coli*. On the *H. influenzae* chromosome *surE* is followed by *lppB*, a homologue of *E. coli* *nlpD*, and a *mutS* gene homologue (encoding a DNA gyrase) (Fig. 6). The arrangement was similar to that observed in a closely related species, *H. somnus*, that has been compared with *E. coli* (Theisen *et al.*, 1993).

In *M. jannaschii*, a *pcm* homologue with 45.6% amino acid identity can be identified. Putative Pcm activity was observed in another thermophilic Archaeon, *Sulfolobus acidocaldarius*, using ovalbumin as the methyl-accepting substrate (Li, 1993). However, no activation could be detected when L-isoaspartyl-containing peptides were included in the reaction. It has not been determined whether the *S. acidocaldarius* enzyme works only on proteins but not on peptide methyl-accepting substrates, or whether the exogenous peptide substrates were rapidly degraded by the bacterial extract and thus could not be methylated by the putative methyltransferase. Nevertheless, since a highly conserved *pcm* gene is present in *M. jannaschii*, it is likely that the enzyme is conserved in the Archaea and might play some role in their survival. A *surE* homologue (34.4% identity) can also be identified in the *M. jannaschii* genome but is far separated from *pcm*.

Recently, using nucleotide sequence comparison analysis, the presence of *pcm* has been proposed in *Pseudomonas aeruginosa* (Tanaka & Takahashi, 1994) and *Thermotoga maritima* (Swanson *et al.*, 1996). However, both sequences lack the upstream region of *pcm* and

thus are insufficient to determine if the *surE*–*pcm* operon arrangement is present in these bacteria. As shown in *H. influenzae*, *pcm* could be totally missing. Our *lacZ* operon fusion results indicate that in *E. coli*, although the *surE* and *pcm* gene overlap by 4 bp, and as well as the *surE* promoter that is responsible for the bicistronic expression of *surE* and *pcm*, a strong promoter is located directly upstream of *pcm* (P3) and the regulation of both genes can be separated.

The level of induction of both *pcmp* and *surEp* from exponential to stationary phase is approximately ten-fold. These results can be compared with the 1.7-fold increase in Pcm activity of strain MC1000 cells from exponential to stationary phase when grown in LB (Li & Clarke, 1992b). It is very likely that post-transcriptional regulation might be important for the expression of *pcm*.

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REFERENCES

- Bremer, H. & Dennis, P. P. (1987). Modulation of chemical composition and other parameters of the cell by growth rate. In *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, pp. 1527–1542. Edited by F. C. Neidhardt, J. L. Ingraham, K. Brooks Low, B. Magasanik, M. Schaechter & H. E. Umbarger. Washington, DC: American Society for Microbiology.
- Brennan, T. V., Anderson, J. W., Jia, Z., Waygood, E. B. & Clarke, S. (1994). Repair of spontaneously deamidated HPr phosphocarrier protein catalyzed by the L-isoaspartate-(D-aspartate) O-methyltransferase. *J Biol Chem* **269**, 24586–24595.
- Bult, C. J., White, O., Olsen, G. J. and 37 other authors (1996). Complete genome sequence of the methanogenic archaeon, *Methanococcus jannaschii*. *Science* **273**, 1058–1073.
- Clarke, S. (1985). Protein carboxyl methyltransferases: Two distinct classes. *Annu Rev Biochem* **54**, 479–506.
- Fleischmann, R. D., Adams, M. D., White, O. and 37 other authors (1995). Whole-genome random sequencing and assembly of *Haemophilus influenzae* Rd. *Science* **269**, 496–512.
- Fraser, C. M., Gocayne, J. D., White, O. and 26 other authors (1995). The minimal gene complement of *Mycoplasma genitalium*. *Science* **270**, 397–403.
- Fu, J., Ding, L. & Clarke, S. (1991). Purification, gene cloning, and sequence analysis of an L-isoaspartyl protein carboxyl methyltransferase from *Escherichia coli*. *J Biol Chem* **266**, 14562–14572.
- Gordia, S. & Gutierrez, C. (1996). Growth-phase-dependent expression of the osmotically inducible gene *osmC* of *Escherichia coli* K-12. *Mol Microbiol* **19**, 729–736.
- Harding, J., Beswick, H. T., Ajiboye, R., Hubby, R., Blakytyn, R. & Rixon, K. C. (1989). Nonenzymatic post-translational modification of proteins in aging. A review. *Mech Ageing Dev* **50**, 7–16.

- Hengge-Aronis, R. (1993).** Survival of hunger and stress: The role of *rpoS* in early stationary phase gene regulation in *E. coli*. *Cell* **72**, 165–168.
- Hengge-Aronis, R. & Fischer, V. (1992).** Identification and molecular analysis of *glgS*, a novel growth-phase-regulated and *rpoS*-dependent gene involved in glycogen synthesis in *Escherichia coli*. *Mol Microbiol* **6**, 1877–1886.
- Ichikawa, J. K., Li, C., Fu, J. & Clarke, S. (1994).** A gene at 59 minutes on the *Escherichia coli* chromosome encodes a lipoprotein with unusual amino acid repeat sequences. *J Bacteriol* **176**, 1630–1638.
- Ingrosso, D., Fowler, A. V., Bleibaum, J. & Clarke, S. (1989).** Sequence of the D-aspartyl/L-isoaspartyl protein methyltransferase from human erythrocytes. *J Biol Chem* **264**, 20131–20139.
- Johnson, B. A., Murray, E. D., Jr, Clarke, S., Glass, D. B. & Aswad, D. W. (1987).** Protein carboxyl methyltransferase facilitates conversion of atypical L-isoaspartyl peptides to normal L-aspartyl peptides. *J Biol Chem* **262**, 5622–5629.
- Johnson, B. A., Ngo, S. Q. & Aswad, D. W. (1991).** Widespread phylogenetic distribution of a protein methyltransferase that modifies L-isoaspartyl residues. *Biochem Int* **24**, 481–487.
- Kagan, R. M. & Clarke, S. (1995).** Protein L-isoaspartyl methyltransferase from the nematode *Caenorhabditis elegans*: genomic structure and substrate specificity. *Biochemistry* **34**, 10794–10806.
- Kolter, R., Siegele, D. A. & Tormo, A. (1993).** The stationary phase of the bacterial life cycle. *Annu Rev Microbiol* **47**, 855–874.
- Lange, R. & Hengge-Aronis, R. (1991).** Identification of a central regulator of stationary-phase gene expression in *Escherichia coli*. *Mol Microbiol* **5**, 49–59.
- Li, C. (1993).** *The physiological functions of the L-isoaspartyl protein methyltransferase and related proteins in bacteria*. PhD thesis, Department of Chemistry and Biochemistry, University of California, Los Angeles, CA, USA.
- Li, C. & Clarke, S. (1992a).** Distribution of an L-isoaspartyl protein methyltransferase in eubacteria. *J Bacteriol* **174**, 355–361.
- Li, C. & Clarke, S. (1992b).** A protein methyltransferase specific for altered aspartyl residues is important in *Escherichia coli* stationary-phase survival and heat-shock resistance. *Proc Natl Acad Sci USA* **89**, 9885–9889.
- Li, C., Ichikawa, J. K., Ravetto, J. J., Kuo, H.-C., Fu, J. C. & Clarke, S. (1994).** A new gene involved in stationary-phase survival located at 59 minutes on the *Escherichia coli* chromosome. *J Bacteriol* **176**, 6015–6022.
- Loewen, P. C. & Hengge-Aronis, R. (1994).** The role of the sigma factor σ^s (KatF) in bacterial global regulation. *Annu Rev Microbiol* **48**, 53–80.
- Matin, A., Auger, E. A., Blum, P. H. & Schultz, J. E. (1989).** Genetic basis of starvation survival in nondifferentiating bacteria. *Annu Rev Microbiol* **43**, 293–316.
- McFadden, P. N. & Clarke, S. (1982).** Methylation at D-aspartyl residues in erythrocytes: possible step in the repair of aged membrane proteins. *Proc Natl Acad Sci USA* **79**, 2460–2464.
- McFadden, P. N. & Clarke, S. (1987).** Conversion of isoaspartyl peptides to normal peptides: implications for the cellular repair of damaged proteins. *Proc Natl Acad Sci USA* **84**, 2595–2599.
- Miller, J. H. (1992).** *A Short Course in Bacterial Genetics: a Laboratory Manual and Handbook for Escherichia coli and Related Bacteria*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Mudgett, M. B. & Clarke, S. (1993).** Characterization of plant L-isoaspartyl methyltransferases that may be involved in seed survival: Purification, cloning, and sequence analysis of the wheat germ enzyme. *Biochemistry* **32**, 11100–11111.
- Mulvey, M. R., Switala, J., Borys, A. & Loewen, P. (1990).** Regulation of transcription of *katE* and *katF* in *Escherichia coli*. *J Bacteriol* **172**, 6713–6720.
- O'Connor, C. M. & Clarke, S. (1985).** Specific recognition of altered polypeptides by widely distributed methyltransferases. *Biochem Biophys Res Commun* **132**, 1144–1150.
- Roszak, D. B. & Colwell, R. R. (1987).** Survival strategies of bacteria in the natural environment. *Microbiol Rev* **51**, 365–379.
- Siegele, D. A. & Kolter, R. (1992).** Life after log. *J Bacteriol* **174**, 345–348.
- Simons, R. W., Houman, F. & Kleckner, N. (1987).** Improved single and multicopy *lac*-based cloning vectors for protein and operon fusions. *Gene* **53**, 85–96.
- Stadtman, E. R. (1988).** Protein modification in aging. *J Gerontol* **43**, B112–B120.
- Swanson, R. V., Sanna, M. G. & Simon, M. I. (1996).** Thermostable chemotaxis proteins from the hyperthermophilic bacterium *Thermotoga maritima*. *J Bacteriol* **178**, 484–489.
- Tanaka, K. & Takahashi, H. (1994).** Cloning, analysis and expression of an *rpoS* homologue gene from *Pseudomonas aeruginosa* PAO1. *Gene* **150**, 81–85.
- Theisen, M., Rioux, C. R. & Potter, A. A. (1993).** Molecular cloning, nucleotide sequence, and characterization of *lppB*, encoding an antigenic 40-kilodalton lipoprotein of *Haemophilus somnus*. *Infect Immun* **61**, 1793–1798.
- Visick, J. E. & Clarke, S. (1995).** Repair, refold, recycle: how bacteria can deal with spontaneous and environmental damage to proteins. *Mol Microbiol* **16**, 835–845.
- Weichart, D., Lange, R., Henneberg, N. & Hengge-Aronis, R. (1993).** Identification and characterization of stationary phase-inducible genes in *Escherichia coli*. *Mol Microbiol* **10**, 407–420.

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