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A New Gene Involved in Stationary-Phase Survival Located at 59 Minutes on the *Escherichia coli* Chromosome

CHUAN LI,[1,2] JEFFREY K. ICHIKAWA,[1] JEFFREY J. RAVETTO,[1] HUNG-CHIH KUO,[2] JUNE C. FU,[1] AND STEVEN CLARKE[1]*

Department of Chemistry and Biochemistry and the Molecular Biology Institute, University of California, Los Angeles, California 90024-1569,[1] and Institute of Medicine, Chung Shan Medical and Dental College, Taichung, Taiwan, Republic of China[2]

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We determined the DNA sequence of a 2,232-bp region immediately upstream of the *pcm* gene at 59 min on the *Escherichia coli* chromosome that encodes an L-isoaspartyl protein methyltransferase with an important role in stationary-phase survival. Two open reading frames of 477 and 1,524 bp were found oriented in the same direction as that of the *pcm* gene. The latter open reading frame overlapped the 5′ end of the *pcm* gene by 4 bp. Coupled in vitro transcription-translation analysis of DNA containing the 1,524-bp open reading frame directly demonstrated the production of a 37,000-Da polypeptide corresponding to a RNA species generated from a promoter within the open reading frame. The deduced amino acid sequence showed no similarity to known protein sequences. To test the function of this gene product, we constructed a mutant strain in which a kanamycin resistance element was inserted at a *Bst*EII site in the middle of its coding region in an orientation that does not result in reduction of Pcm methyltransferase activity. These cells were found to survive poorly in stationary phase, at elevated temperatures, and in high-salt media compared with parent cells containing the intact gene, and we thus designate this gene *surE* (survival). *surE* appears to be the first gene of a bicistronic operon also containing the *pcm* gene. The phenotypes of mutatations in either gene are very similar and indicate that both gene products are important for the viability of *E. coli* cells under stressful conditions.

In recent years, a number of genes have been found to play roles in maintaining the viability of gram-negative bacteria such as *Escherichia coli* under conditions in which cell growth is not possible (16, 20, 28, 31). These genes include a number that are specifically induced in stationary phase by the KatF/RpoS sigma factor and other products (11, 21, 26, 36, 39). Other proteins appear to be synthesized during both exponential- and stationary-phase growth. One of these constitutively expressed genes encodes a methyltransferase that catalyzes the transfer of the methyl group from *S*-adenosylmethionine to the carboxyl group of abnormal L-isoaspartyl residues originating from the spontaneous degradation of aging proteins (6, 18, 19). This enzyme [protein-L-isoaspartate-(D-aspartate) *O*-methyltransferase; EC 2.1.1.77] has been identified in a broad spectrum of organisms and has been postulated to play a role in repair or degradation pathways that metabolize polypeptides containing these damaged residues (13, 14, 22, 23, 27).

In *Escherichia coli*, the *pcm* gene encoding the L-isoaspartyl protein methyltransferase has been cloned, sequenced, and mapped to the 59-min region of the chromosome in a position separated by one gene from the *katF*/*rpoS* gene (6, 12). *E. coli pcm* mutants grow normally in exponential phase but do not survive well in stationary-phase culture or when exposed to high temperatures (19). These results are consistent with the proposed function of the enzyme in processing abnormal polypeptides that can accumulate with cell aging.

Since a clear promoter could not be identified by sequence similarity to the σ^{70} consensus sequence in the DNA sequence immediately upstream of the constitutively expressed *pcm* gene, we suspected that its promoter might be further upstream and that the *pcm* gene could be present in an operon (6, 19). Because genes of related functions are often located within an operon, it is possible that the gene or genes immediately upstream of *pcm* are also involved in the metabolism of damaged proteins. We obtained preliminary evidence for an operon structure by noting that the insertion of a kanamycin resistance element at a *Bst*EII site 348 bp upstream of the initiation codon of the *pcm* gene resulted in the reduction of methyltransferase activity to 18% of that seen in control cells (19). In this construct, the direction of transcription of the kanamycin resistance *neo* gene was in the opposite direction of that of the *pcm* gene.

We thus sequenced the DNA up to 2.6 kb upstream of the *pcm* gene. We report here our finding of a new gene (*surE*) that appears to be transcribed as the first gene in a bicistronic operon with the *pcm* gene. Construction of a mutant by inserting a kanamycin resistance (Km^r) element in *surE* in the same orientation as that of the *pcm* gene did not decrease Pcm methyltransferase levels. However, the ability of the mutants to survive in stationary phase was still greatly diminished. Comparisons of the deduced amino acid sequence of SurE with other sequences do not reveal any similarities with previously described proteins. These results thus define a new type of protein that plays a role in the survival of *E. coli* cells under suboptimal conditions.

MATERIALS AND METHODS

Bacterial strains and plasmids. The *E. coli* strains and plasmids used in this study are listed in Table 1. Plasmid DNA used in sequencing or coupled in vitro transcription-translation reactions was prepared by column chromatography as instructed by the manufacturer (Qiagen).

DNA sequencing. Both strands of the DNA sequences of the 2.3-kb *Bam*HI-*Bst*EII DNA fragment upstream of the *pcm*

* Corresponding author. Mailing address: Department of Chemistry and Biochemistry, UCLA, 405 Hilgard Ave., Los Angeles, CA 90024-1569. Phone: (310) 825-8754. Fax: (310) 206-7286. Electronic mail address: clarke@ewald.mbi.ucla.edu.

TABLE 1. *E. coli* strains and plasmids used

Strain or plasmid	Description	Reference or source
Strains		
MC1000	F⁻*araD139* Δ(*araABC-leu*)*7679 galU galK* Δ(*lacX*)*74 rpsL thi*	1
CL1010	MC1000, Δ*pcm*(Δ*Mlu*I-*Cla*I)::Km^r	19
CL2010	MC1000, *orf1* (*surE*)::Km^r (Km^r element transcribed in the opposite direction from that of *surE* and *pcm*)	19
CL4010	MC1000, *glnG*::Km^r	19
JKI2010	MC1000, *orf1 surE*)::Km^r (Km^r element transcribed in the same direction as that of *surE* and *pcm*)	This study
JC7623	F⁻ *thr-1 leu-6 proA2 his-4 thi-1 argE3 lacY1 galK2 ara-14 xyl-5 mtl-1 tsx-33 rpsL31 supE37 recB21 recC22 sbcB15 sbsC201*	40
Plasmids		
pMMkatF1	10.2-kb *Bam*HI *E. coli* chromosomal fragment in pAT153	P. Loewen (25)
pCL1	Religation of a 7.5-kb *Eco*RI-*Sna*BI fragment from pMMkatF1 (contains the entire *orf0*, *surE*, and *pcm* genes and the 5′ portion of the *nlpD* gene)	19
pCL2	4.5-kb *Bam*HI-*Bsp*HI fragment from pMMkatF1 insert in the *Bam*HI-*Sma*I polylinker site of pGEM-7Zf (+) (Promega) (contains the entire *orf0*, *surE*, *pcm*, and *nlpD* genes and the 5′ portion of the *katF*/*rpoS* gene)	12
pJF10	2.4-kb *Eco*RV fragment from pMMkatF1 insert in pUC19 (contains the entire *surE* gene and flanking regions)	6
pJKI20	1.2-kb Km^r cassette (*Sma*I fragment of pUC4-KIXX) inserted into the *Bst*EII site of pJF10 with the kanamycin resistance gene transcribed in the same direction as the *surE* gene)	This study

gene at 59 min on the *E. coli* chromosome were determined by either manual dideoxy reactions using primer walking with the Sanger reaction on DNA inserts from plasmids pMMkatF1 and pCL1 as described previously (12) or automatically for the inserts in the pUC19-derived plasmids pJF10 and pJF34 as described previously (6). Oligonucleotide primers of 20 nucleotides were synthesized according to the initial sequences obtained.

Construction of mutants be gene replacement. We disrupted the chromosomal copy of the gene on the 5′ end of *pcm* by inserting a Km^r cassette into the *Bst*EII site. Plasmid pJF10, containing this gene and its flanking regions (Table 1), was digested at its unique *Bst*EII site and blunt ended by using the Klenow fragment of DNA polymerase. Plasmid pUC4KIXX (Pharmacia) was digested with *Sma*I to produce a 1.4-kb Km^r cassette that was gel purified by using Magic PCR Preps (Promega). We then ligated the Km^r cassette into the blunt-ended *Bst*EII site of pJF10. The orientation of the Km^r cassette in a number of clones was determined by analysis of fragment lengths after restriction endonuclease cleavage with *Bam*HI, *Bgl*II, *Pst*I, and *Sph*I. We selected one clone (pJKI20) in which the direction of transcription of the Km^r element was in the same direction as that of the *pcm* gene. Plasmids purified from these cells were then used to transform *E. coli* JC7623 by the method described by Chung et al. (3). Cells were selected for double recombination events of the plasmid and the chromosome in which ampicillin resistance was lost and kanamycin resistance was retained. A P1 phage lysate was prepared from these cells and used to infect MC1000 cells by the protocol of Silhavy et al. (33). Chromosomal DNA from the kanamycin-resistant P1 transductants was digested with *Bam*HI and *Eco*RV and analyzed by Southern blot analysis with a ^{32}P-end-labeled 20-bp oligonucleotide corresponding to nucleotides 1900 to 1919 of the sequence shown in Fig. 1. One MC1000 progeny with hybridizing fragments 1.2 kb larger than those from the parent strain (indicating the presence of the 1.2-kb Km^r cassette in the homologous chromosomal location) was designated strain JKI2010.

Analysis of methyltransferase levels in cell extracts. Cells (500 ml) were grown overnight with shaking at 37°C in Luria-Bertani (LB) medium with the addition of 100 μg of kanamycin per ml for the mutant strains. Cells were pelleted by centrifugation at 4°C, washed in buffer A (5 mM sodium phosphate, 5 mM sodium EDTA, 10% [wt/vol] glycerol, 25 μM phenylmethanesulfonyl fluoride, 15 mM β-mercaptoethanol [pH 7.0]), resuspended in 5 ml of buffer A, and lysed by two rounds of French press treatment at 16,000 lb/in^2. The extract was centrifuged at 16,000 × *g* for 20 min at 4°C to remove cell debris and unbroken cells. The protein concentration of the soluble lysate was determined by a modified Lowry procedure after protein precipitation with 10% trichloroacetic acid. The methyltransferase activity was assayed in a 40-μl reaction mixture containing 100 to 200 μg of protein, 10 μM *S*-adenosyl-L-[*methyl*-^{14}C]methionine (52 mCi/mmol; ICN), and 0.1 M sodium citrate (pH 6.0). When added, the L-isoapartyl-containing peptide substrate (L-Lys-L-Ala-L-Ser-L-Ala-L-isoAsp-L-Leu-L-Ala-L-Lys-L-Tyr) was used at a final concentration of 100 μM. Samples were incubated for 20 min at 37°C, and the base-labile, volatile [^{14}C]methanol radioactivity resulting from methyl ester hydrolysis was quantitated as described previously (6).

Analysis of stationary-phase heat shock and survival. Long-term survival of *E. coli* strains was determined as described previously (19), with the following modifications. Cells were incubated at 250 rpm in a New Brunswick Innova air incubator. Serial dilutions were made in M9 salts (24). Kanamycin-resistant cells were plated on LB plates containing 100 μg of kanamycin per ml. Heat shock survival was measured as described previously (19) except that an aliquot of the overnight culture was diluted 1,000-fold into 0.9% (wt/vol) sodium chloride preheated to 55°C in 50-ml polypropylene centrifuge tubes. At each time point, aliquots of 10 or 100 μl were diluted into 10 ml of 0.9% sodium chloride at room temperature. The diluted suspensions (100 and 200 μl) were plated as described above.

Nucleotide sequence accession number. The nucleotide sequence determined in this study has been deposited in the GenBank database under accession number L07942.

RESULTS

DNA sequence up to 2.6 kb upstream of the *pcm* gene. Both strands of the DNA upstream of the *pcm* gene in the inserts of plasmid pMMkatF1 were sequenced. Starting from the *Bam*HI

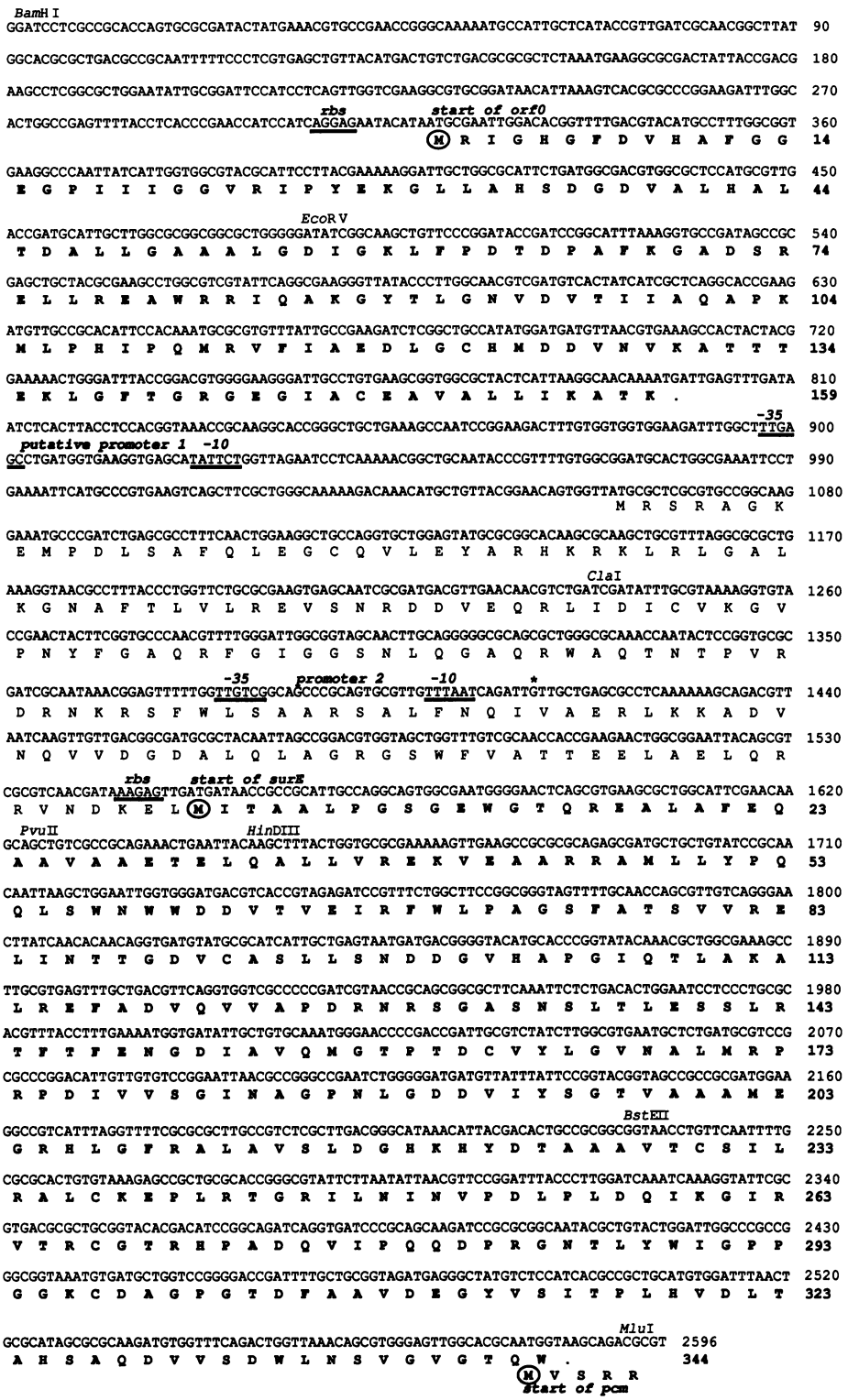

FIG. 1. Nucleotide and deduced amino acid sequences of the *E. coli* chromosome in the region upstream of the *pcm* gene. The numbers on the right indicate the last base at the end of the row starting from the *Bam*HI site at about kb 2888.6 on the *E. coli* EcoMap6 physical map (15, 29). The DNA sequence from the *Bst*EII site to the *Mlu*I site has been published by Fu et al. (6). The positions recognized by the restriction enzymes *Bam*HI, *Eco*RV, *Cla*I, *Pvu*II, *Hin*dIII, *Bst*EII, and *Mlu*I are indicated. Putative promoters derived from the consensus sequences of the recognition site of the σ^{70} RNA polymerase are indicated, with the −10 and −35 regions labeled. The experimentally determined transcriptional start site in promoter 2 derived from primer extension studies is marked with an asterisk. Open reading frames expressed in in vitro transcription-translation experiments are shown in boldface. Potential Shine-Dalgarno regions for ribosome binding are shown. Nucleotides are numbered at the right from the first base in the *Bam*HI site; amino acids are numbered for the *orf0* product and SurE (in boldface).

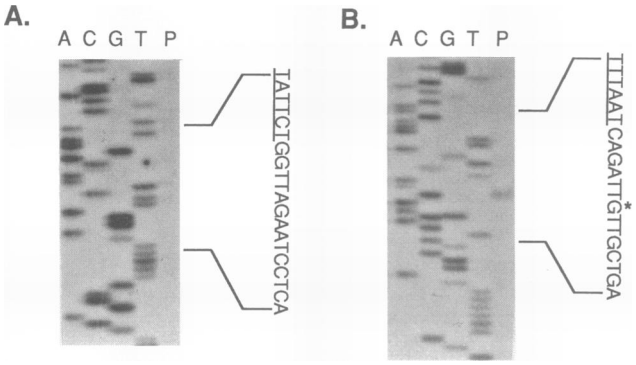

FIG. 2. Primer extension analysis of 5′ mRNA termini. The preparation of total RNA from *E. coli* and the procedures for primer extension reaction are described by Ichikawa et al. (12). The results of using primers complementary to the regions around 70 to 90 bp downstream of the −10 region of putative promoters P1 (5′-AGCT GACTTCACGGGCATGA) and P2 (5′-CGACAAACCAGCTAC CACGT) (Fig. 1) are shown in panels A and B, respectively. For each panel, a portion of the DNA sequence of the sense strand starting from the −10 region of the putative promoters is illustrated. The ACGT label on each lane indicates the terminating dideoxyribonucleotide included in the sequencing reaction. The product of the primer extension reaction was electrophoresed in lane P. The G nucleotide residue determined to be the transcriptional start site in panel B is indicated by an asterisk. In each case, no other signals corresponding to start sites were detected for up to 150 bp upstream of each region shown here.

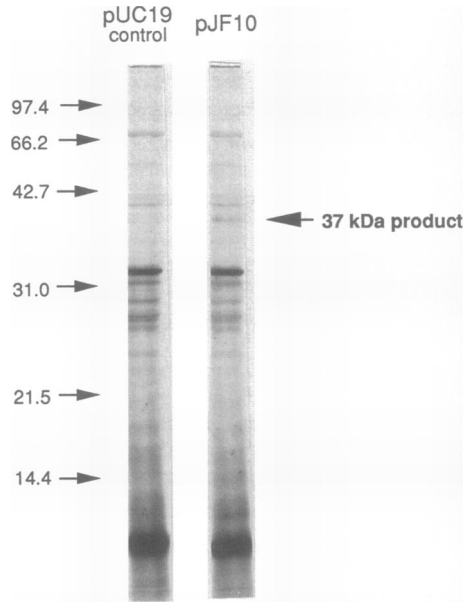

FIG. 3. In vitro transcription-translation of the gene adjacent to the 5′ end of the *pcm* gene at 59 min on the *E. coli* chromosome. Analysis of the ^{35}S-labeled polypeptides in an in vitro system by Laemmli gel electrophoresis in sodium dodecyl sulfate (12.5% acrylamide gel) followed by staining with Coomassie brilliant blue, destaining, and drying the gel for autoradiography was performed as described by Ichikawa et al. (12). The template plasmid included in each reaction mixture is indicated above each lane. The arrow on the right points to the position of the 37,000-Da gene product specified by the chromosomal DNA insert on the plasmid pJF10. Positions of protein molecular weight standards (BioRad low-molecular-weight standards, including rabbit muscle phosphorylase *b* [97,400], bovine serum albumin [66,200], hen egg white ovalbumin [42,699], bovine carbonic anhydrase [31,000], soybean trypsin inhibitor [21,500], and hen egg white lysozyme [14,400]) are marked on the left in thousands.

site at kb 2888.6 on the *E. coli* EcoMap6 physical map (15, 29), we sequenced 2.232 kb to the *Bst*EII site at approximately kb 2886.4 on the physical map at the 5′ boundary of the previously reported *pcm* gene and flanking regions (6) (Fig. 1). A *Cla*I site that had not been detected from restriction endonuclease mapping (6, 25) is found at positions 1237 to 1242 in the DNA sequence. The failure of *Cla*I to cut at this position is probably due to the presence of an overlapping GATC sequence at positions 1236 to 1239 that is recognized by the endogenous *dam* methyltransferase (2).

Identification of the protein product of a new gene overlapping the *pcm* gene. Analysis of the DNA sequence in Fig. 1 reveals the presence of an open reading frame beginning at nucleotide 1060 and extending 1,524 bp to overlap with the *pcm* gene by 4 bp at its 5′ end. Both this open reading frame and the *pcm* gene are oriented 5′ to 3′ counterclockwise on the *E. coli* chromosome. Comparison of the DNA sequence in the region of this open reading frame with σ^{70} promoter consensus sequences (10) suggested the two possible promoters indicated in Fig. 1. Using primer extension analysis, we detected no evidence for transcripts from putative promoter 1 located about 130 bp upstream of the 1,524-bp open reading frame but obtained clear evidence for a single start site of transcription from promoter 2 located in the 5′ region of the open reading frame (Fig. 2).

The determination of a promoter at site 2 suggests that translation of the 1,524-bp open reading frame begins at an ATG codon about 140 bp downstream of the transcriptional start site with an AAGAG Shine-Dalgarno sequence (34). In this case, the product of the open reading frame would be of 344 amino acid residues instead of the maximal 508 amino acid residues, with a calculated molecular weight of 36,955 instead of 55,428. We performed coupled in vitro transcription-translation experiments to determine which polypeptide is the actual product. A pUC19-derived plasmid pJF10 was used as the template DNA. This plasmid contains a 2.4-kb *Eco*RV insert with the complete open reading frame of the gene on the 5′ end of the *pcm* gene with flanking regions corresponding to nucleotides 494 on Fig. 1 to a position in the middle of the *pcm* coding region (6). As shown in Fig. 3, we detected only one polypeptide of about 37 kDa which was absent when pUC19 DNA was used as a template in place of pJF10. Similar results were obtained from maxicell experiments that analyze the plasmid-coded polypeptides (reference 30 and data not shown). This result is consistent with the activity of a promoter at site 2.

Possible homology of the 37-kDa polypeptide with other proteins. The amino acid sequence of the 37-kDa protein predicts a polypeptide with an isoelectric point of about 4.9. No extended stretches of hydrophobic residues consistent with membrane-spanning regions are detected. Searches of the nonredundant PDB + SwissProt + PIR + SPUpdate + GenPept + GPUpdate database at the National Center for Biotechnology Information, using the BLAST network service on 13 December 1993, were made with the six translated reading frames of the DNA sequence shown in Fig. 1. This analysis revealed no proteins with significant homologies. However, translation of DNA from the 5′ flanking region of the *lppB* gene of *Haemophilis somnus* (37) revealed 49% identity in a 49-amino-acid overlap with the C-terminal region of the deduced 37-kDa *E. coli* polypeptide, suggesting that its homolog is present in this

TABLE 2. L-Isoapartyl methyltransferase activities in extracts of *E. coli* strains

Strain	Phenotype	Methyltransferase activity[a] (pmol/min/mg of protein) ± SD		% of control[b]
		Endogenous methyl-accepting substrates	L-isoaspartyl peptide	
MC1000	SurE$^+$ Pcm$^+$	0.94 ± 0.25	4.40 ± 0.64	100
CL1010	SurE$^+$ Pcm$^-$	0.17 ± 0.03	0.17 ± 0.03	0
CL2010	SurE$^-$ Pcm$^+$	0.20 ± 0.07	0.67 ± 0.10	14
JKI2010	SurE$^-$ Pcm$^+$	0.61 ± 0.22	8.13 ± 0.22	217

[a] Measured as described in Materials and Methods.
[b] Measured in comparison to MC1000 strain, using the L-isoaspartyl-specific activity determined by substracting the endogenous activity from the activity in the presence of the L-isoaspartyl peptide substrate.

bacterium; translation of additional 5′ DNA sequence from *H. somnus* may reveal additional similarities with the N-terminal and central regions of the *E. coli* polypeptide.

Mutant analysis. Previously, a mutant strain of *E. coli* (CL2010) was constructed by inserting a Km^r cassette at the *Bst*EII site in the middle of the open reading frame preceding the *pcm* gene in the opposite transcriptional orientation (19). We found that stationary-phase survival was decreased in this strain, although it was not clear whether this was a direct result of the mutation to this gene or of a polar effect on Pcm methyltransferase activity, which was reduced to 18% of the control value in this strain (19). To obtain a mutant strain in which the Pcm activity was maintained, we inserted the kanamycin resistance gene at this site in the same orientation as that of the open reading frame and the *pcm* gene to obtain strain JKI2010. As shown in Table 2, this strain demonstrated a methyltransferase activity about twofold higher than that of the control cells, suggesting that the *neo* gene promoter-derived transcripts include the *pcm* message. As controls, we showed that a *pcm* deletion strain (CL1010) had no L-isoaspartyl-specific methyltransferase activity, while we confirmed the low methyltransferase activity (14% of the control level) of strain CL2010 (Table 2).

We compared the abilities of strains JKI2010 and its wild-type parent MC1000 to survive in stationary phase. As shown in Fig. 4, after 10 days in stationary phase, 40% of wild-type cells could still form colonies, but only 5% of the mutant cells were able to do so. The survival of the mutant cells was found to be similar to that of cells in which the *pcm* gene was deleted (strain CL1010; Fig. 4). These results indicate that disruption of the 37-kDa polypeptide in itself is deleterious for stationary-phase survival.

We then tested the abilities of these cells to survive a 55°C heat challenge (Fig. 5). After 12 min of this treatment, about 35% of the parent MC1000 cells could form colonies, whereas only 0.2 to 0.6% of either the mutant JKI2010 or CL2010 cells were capable of doing so. Again, the survival of these mutants was similar at each time point to that of the CL1010 mutant containing the *pcm* gene deletion. Control experiments were performed to determine whether the presence of the Km^r cassette itself might itself lead to heat sensitivity. We found, however, that CL4010 cells, containing a Km^r element in the unrelated *glnG* gene, demonstrated heat resistance similar to that of the parent MC1000 cells (Fig. 5).

Finally, we tested the viabilities of these mutants when osmotically stressed (Fig. 6). We found that while about 80 to 90% of the parent MC1000 cells were capable of forming colonies after 2 to 3 h in 2.5 M sodium chloride, less than 15%

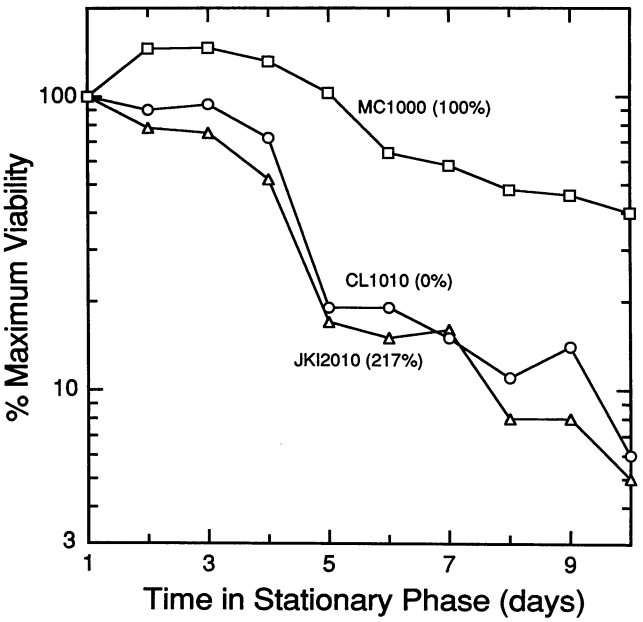

FIG. 4. Stationary-phase survival. Parent cells (MC1000), a derivative containing a Km^r element inserted into the open reading frame on the 5′ end of the *pcm* gene (JKI2010), and a derivative in which the *pcm* gene was largely replaced by a Km^r element (CL1010) were incubated at 37°C in minimal medium as described in Materials and Methods. After the indicated time in stationary phase, aliquots were removed and plated on LB agar plates to determine the number of viable colonies. Results are expressed as the percentage of viable colonies at each time point compared with the number of colonies seen after 1 day in stationary phase. The value in parentheses after the strain name gives the relative level of Pcm methyltransferase activity (Table 2). The data shown represent average values from four separate experiments.

of the mutant cells with the disrupted 37-kDa polypeptide or the Pcm methyltransferase were similarly capable. Again, the presence of the Km^r cassette in strain CL4010 did not result in increased osmotic sensitivity compared with the parent MC1000 cells (Fig. 6).

These results suggest that the gene on the 5′ end of the *pcm* gene encodes a protein essential for stationary-phase survival and for survival of heat and osmotic stresses. We thus designate this gene *surE*, in accordance with the nomenclature of Tormo et al. (38) and Siegele and Kolter (32).

Identification of a new gene on the 5′ end of *surE*. The DNA sequence analysis in Fig. 1 shows the presence of an additional open reading frame of 477 bp that is separated by 752 bp from the 5′ coding end of the *surE* gene. This open reading frame can potentially encode a protein of 159 amino acid residues beginning with a methionine-encoding ATG codon 319 bases downstream of the *Bam*HI site. Coupled transcription-translation experiments using template DNA from pCL2 (Table 1) revealed that a 16,900-Da product corresponding to the 159 amino acid residues was produced (12). The 16.9-kDa polypeptide is well expressed in the in vitro system, suggesting that sufficient promoter elements are present 3′ of the *Bam*HI site. A good Shine-Dalgarno sequence for translation is present (AGGAG); however, we were not able to identify a specific promoter sequence. Sequence analysis suggests that this polypeptide has an isoelectric point of about 6.3 and that it lacks stretches of hydrophobic residues that may be membrane spanning. Even though the overall distribution of polar and

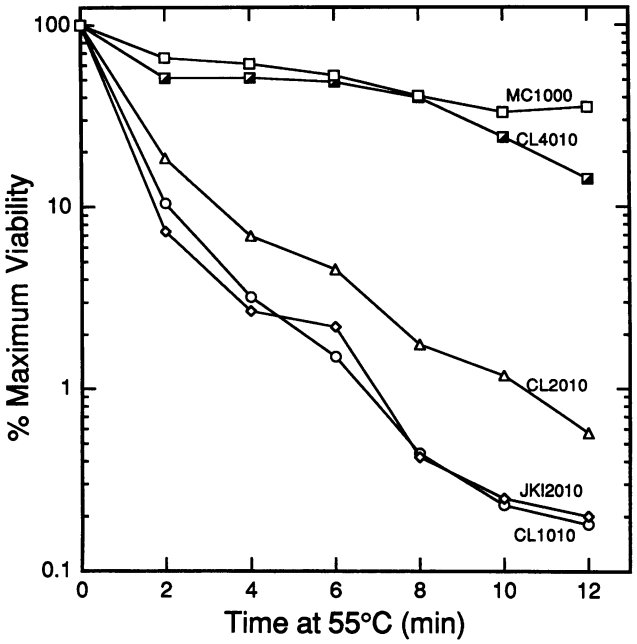

FIG. 5. Heat shock survival. Parent cells (MC1000), derivatives containing a Km^r element inserted into the open reading frame on the 5′ end of the *pcm* gene (CL2010 and JKI2010), a derivative in which the *pcm* gene was largely replaced by a Km^r element (CL1010), and a derivative with a Km^r element in the *glnG* gene (CL4010) were incubated at 55°C in 0.9% (wt/vol) sodium chloride as described in Materials and Methods. Viable colony counts were determined after plating on LB agar plates and compared at various times with the number at zero time (no heating). The data shown represent average values from eight experiments.

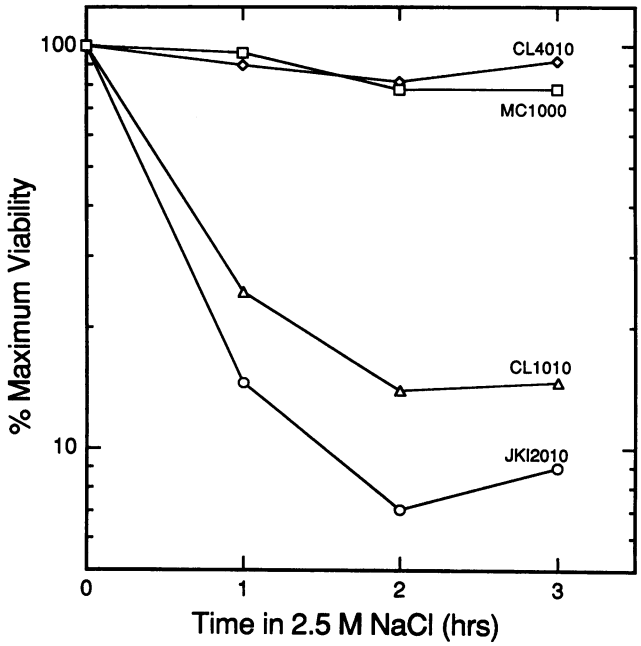

FIG. 6. Survival to osmotic stress. Parent cells (MC1000), a derivative containing a Km^r element inserted into the open reading frame on the 5′ end of the *pcm* gene (JKI2010), a derivative in which the *pcm* gene was largely replaced by a Km^r element (CL1010), and a derivative with a Km^r element in the *glnG* gene (CL4010) were grown in M9 medium to stationary phase and were then incubated at 37°C in 2.5 M sodium chloride for the indicated times. Viable colony counts were determined after plating on LB agar plates and compared at various times with the number of colonies not osmotically stressed. The data shown represent average values from two experiments.

nonpolar amino acid residues is not exceptional, the protein has an unusually low number of serine, asparagine, and glutamine residues (two, two, and three, respectively). A search for similar sequences by using the BLAST network service at the National Center for Biotechnology Information revealed two highly homologous species of the 159-amino-acid deduced protein. One of these is an open reading frame in *Rhodobacter capsulatus* upstream of the *nifR3* nitrogen regulatory gene that has 48% sequence identity over 157 residues (5). The other is an open reading frame in *Bacillus subtilis* immediately upstream of the *gltX* glutamyl-tRNA synthetase gene, with 63% sequence identity over 157 residues (7). Interestingly, the *E. coli* gene is located about 1 kb downstream of the *cysC* gene in the *cysCDN* operon (17), while the *B. subtilis* homolog is separated from the *cysES* operon by the *gltX* gene (7). These sequences are aligned in Fig. 7. No information is available on the function of any of these three genes, but the high degree of conservation of sequence in these gram-positive and gram-negative bacteria suggests a central function of their products.

DISCUSSION

We have presented evidence for a new gene (*surE*) involved in stationary-phase survival at 59 min on the *E. coli* chromosome. We show that disruption of this gene not only results in dramatically reduced survival rates in stationary phase but also increases sensitivity to heat and osmotic stresses. The biochemical function of the 37-kDa polypeptide encoded by the *surE* gene is unknown; comparisons of its amino acid sequence with other known protein sequences did not reveal any structural relationships. One possible clue to the function of the *surE* gene comes from evidence that this gene is present as the first element in a bicistronic operon with the *pcm* gene. These genes are transcribed in the same direction, and their coding regions overlap by 4 bp. We show that insertion of a Km^r element in the *surE* gene can have polar effects on the *pcm* gene, either increasing or decreasing methyltransferase activity depending on the direction of the insertion (Table 2). We also used reverse transcriptase and PCR to show that RNA species are synthesized containing elements of both the *surE* and *pcm* genes (data not shown). These results suggest that the functions of the *surE* and *pcm* genes may be related to each other. Since the methyltransferase encoded by *pcm* is known to modify atypical L-isoaspartyl residues that have been spontaneously generated from normal L-aspartyl or L-asparaginyl residues (4), the product of *surE* might also be involved in the metabolic pathways that can reduce the time-dependent accumulation of proteins containing altered aspartyl residues.

The phenotypes of *surE* and *pcm* mutants are very similar. The two mutants demonstrate similar degrees of loss of viability during extended times in stationary phase, 55°C heat treatment, or osmotic stress in 2.5 M sodium chloride. Additionally, both mutants show normal logarithmic-phase rates of growth in rich and minimal media (19). The stationary-phase and heat sensitivity phenotypes have been explained for *pcm* mutants in terms of the effects of the accumulation of damaged proteins containing L-isoaspartyl residues. These altered proteins may be able to function during nonstress conditions but

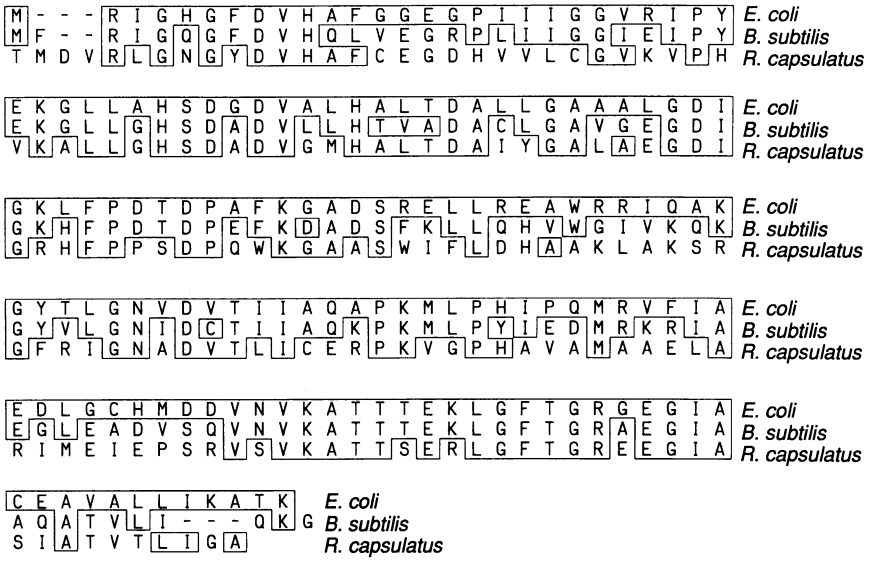

FIG. 7. Aligment of deduced amino acid sequences of open reading frames from *B. subtilis* (GenBank entry L14580 [7]) and *R. capsulatus* (*orf2* [5]) with with sequence of *orf0* gene described here. Identical residues are boxed.

may be more sensitive to denaturation at 37°C over extended periods and at 55°C for even short times (19). We are now interested in determining how *surE* mutants might exhibit similar characteristics. We have considered the possibility that the *surE* gene product represents a previously described peptidase that cleaves L-isoaspartyl-containing dipeptides in *E. coli* (9). The loss of such an enzyme may prevent the full degradation of proteins containing isoaspartyl residues and result in cell toxicity due to accumulated substrate. However, we have shown that no loss of this activity occurs in strain CL2010, in which the *surE* gene is disrupted by Km^r cassette at the *Bst*EII site (8).

It is interesting that four adjacent genes in the 59-min region of the *E. coli* chromosome are all involved in aspects of stationary phase survival (Fig. 8) (12). These genes (*surE-pcm-nlpD-katF/rpoS*) are all transcribed in a counterclockwise direction, and the *surE* and *pcm* genes appear to be present in an operon. The short intragenic spacing of the *pcm* and *nlpD* genes (141 bp) and the *nlpD* and *katF/rpoS* genes (64 bp) (12) suggests that these genes may have been part of an ancestral operon involved in stationary phase survival.

Recent DNA sequencing studies have revealed similarities in the organization of the genomes of *Pseudomonas aeruginosa* and *H. somnus* with the 59-min region of *E. coli*. For example, full homologs of the *E. coli katF/rpoS* and the *nlpD* lipoprotein genes and the 3′ end of the *pcm* gene have been detected in a 2,910-bp section of *P. aeruginosa* DNA (35). In a 1,815-bp segment of *H. somnus* DNA (37), a nearly complete homolog of the *nlpD* gene is found (*lppB*) flanked by an unidentified *orfX* and the 3′ portion of a *surE* homolog. These relationships are shown in Fig. 8. Although these results suggest a conservation of gene order in this segment of the chromosome in these bacterial species, the *orfX* gene in *H. somnus* is not related to the *pcm* gene in *E. coli* or *P. aeruginosa* and does not

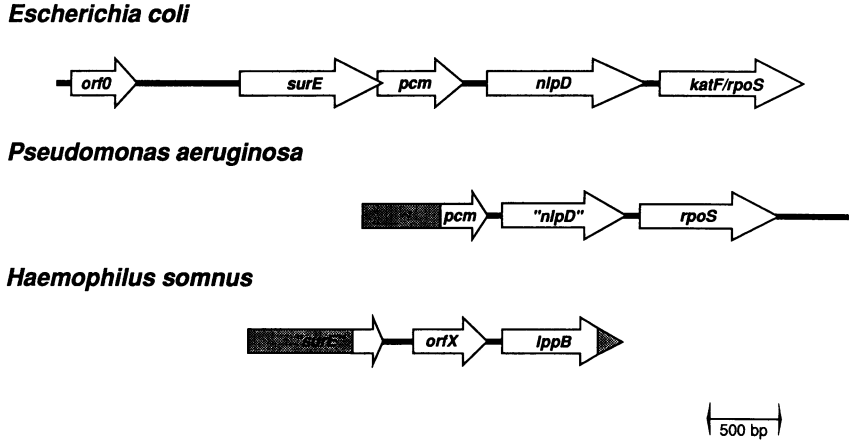

FIG. 8. Organization of bacterial chromosomes containing genes related to those adjacent to the *pcm* gene on the *E. coli* chromosome at 59 min. Genes were identified from nucleic acid sequence analysis in *P. aeruginosa* (GenBank entry D26134 [35]) and *H. somnus* (37). Open reading frames are shown as open arrows; dotted portions represent homologous areas for which no sequence information is available. There appears to be no sequence relationship between the *orfX* gene of *H. somnus* and the *pcm* genes of *E. coli* and *P. aeruginosa*.

appear to be present in an operon with the *surE* homolog. The Pcm L-isoaspartyl methyltransferase activity has been detected in a wide variety of gram-negative bacteria, including *P. aeruginosa* (18), but it is unclear whether its gene is not present in *H. somnus* or whether it is located in a different part of the chromosome.

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