JOURNAL OF BACTERIOLOGY, Aug. 1998, p. 4287–4290 Vol. 180, No. 16
0021-9193/98/$04.00+0

NOTES

The *Escherichia coli* Starvation Gene *cstC* Is Involved in Amino Acid Catabolism

C. D. FRALEY,† J. H. KIM,‡ M. P. MCCANN,§ AND A. MATIN*

Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, California 94305

Received 14 January 1998/Accepted 3 June 1998

***Escherichia coli* strains mutant in the starvation gene *cstC* grow normally in a mineral salts medium but are impaired in utilizing amino acids as nitrogen sources. They are also compromised in starvation survival, where amino acid catabolism is important. The *cstC* gene encodes a 406-amino-acid protein that closely resembles the *E. coli* ArgD protein, which is involved in arginine biosynthesis. We postulate that CstC is a counterpart of ArgD in an amino acid catabolic pathway. The *cstC* upstream region contains several regulatory consensus sequences. Both σ^S and σ^{54} promoters are probably involved in *cstC* transcription and appear to compete with each other, presumably to match *cstC* expression to the cellular amino acid catabolic needs.**

Escherichia coli differentiates into a resistant cellular state in response to starvation due to the expression of 30 to 80 starvation genes (12–14). We report here on the role of an *E. coli* starvation gene, *cstC* (map position, 38.2 min) that we described previously (2, 9).

Bacterial strains and plasmids used are listed in Table 1. Cultures were grown in Luria-Bertani broth or in M9 supplemented with D-glucose as described previously (10). All experiments were done at least twice.

***cstC* is involved in amino acid catabolism.** The *cstC-lacZ* transcriptional fusion strain, AMS96, demonstrated wild-type growth in LB or glucose-M9 medium, but it was impaired in using amino acids as sole nitrogen sources. While the wild type had doubling times of 4.5 h with L-ornithine and 7.5 h with *N*-α-acetyl-L-ornithine or L-arginine as nitrogen source, the doubling time of the mutant in glucose-L-ornithine medium was 23 h, and it did not grow with L-arginine as nitrogen source. The mutant was also impaired in starvation survival, where amino acid catabolism is important (3): at 125 h after the exhaustion of ammonium from glucose-M9 medium, the wild-type culture showed 60% viability, but AMS96 showed only 4% viability.

To further explore if the *cstC* gene in fact had a role in amino acid catabolism, the gene and contiguous region (Fig. 1) were cloned from the Kohara *E. coli* miniset collection, using 1.6-kb *Pst*I-*Bgl*II ^{32}P-labeled fragment originally from pAMC3 (2) as probe. The desired DNA was obtained from phage 328 and cloned into pBluescript II KS(+), generating pAMC162. Sequencing of the *Pst*I and *Xmn*I region (Fig. 1) showed that the 5′ end of the cloned fragment corresponded to nucleotide (nt) 99 of the *xthA* gene (19), which is transcribed divergently to *cstC*. A putative open reading frame (ORF)

TABLE 1. *E. coli* strains and plasmids used in this study

Strain or plasmid	Genotype	Source or reference
E. coli strains[a]		
K-12	Wild type (λ^- F^-)	Laboratory stock; 2
MC4100	F^- *araD139 rpsL150* Δ(*argF-lac*)*U169 relA1 ptsF25 flbB5301 deoC1 rbsR* (*thiA*?)	Laboratory stock; 2
YMC18	K-12 strain 294 (*endA1 thi-1 hsdR17* [r_K^- m_K^+]) *supE44 hutC*$_{Klebs}$[b] Δ(*lac*)*U169 rpoN208*::Tn*10*	23
AMS6	K-12 and Δ(*lac*)*U169*	21
AMS35	MC4100 *cstC*::Mu dX(*lac* Ap^r Tn*9*)	2
AMS96	AMS6 *cstC*::Mu dX(*lac* Ap^r Tn*9*)	2
AMS150	K-12 and *rpoS*::Tn*10*	15
AMS349	K-12 and *cstC*::*kan*	This study
AMS351	AMS96 and *rpoN208*::Tn*10*	This study
AMS352	AMS96 and *rpoS*::Tn*10*	This study
Plasmids		
pBluescript II KS(+)		Stratagene, Inc.
pAMC3	pBW2, modified by deletion of the anti-*tet* promoter (P1) region, with an ~11.2-kb AMS35 *Pst*I fragment containing the *cstC* region	2
pAMC162	pBluescript II KS(+) with an ~4-kb *Pst*I-*Eco*RI fragment containing the *cstC* region from the Kohara miniset collection phage 328	This study

[a] Standard phage $P1_{vir}$ protocols were used to construct strains for this study.
[b] Mutation from *Klebsiella*.

* Corresponding author. Mailing address: D317 Sherman Fairchild Science Building, Department of Microbiology & Immunology, Stanford University School of Medicine, Stanford, CA 94305-5402. Phone: (650) 725-4745. Fax: (650) 725-6757. E-mail: a.matin@forsythe.stanford.edu.

† Present address: Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305-5307.

‡ Present address: Department of Food Science & Technology, College of Agriculture, Gyeongsang National University, Chinju, Korea 660-701.

§ Department of Biology, St. Joseph's University, Philadelphia, PA 19131.

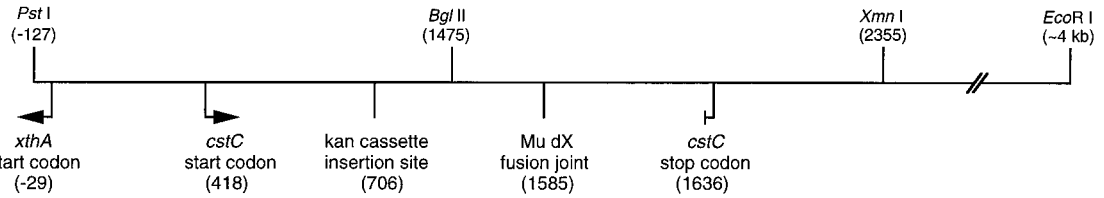

FIG. 1. The *Pst*I-*Eco*RI fragment containing the *cstC* gene cloned from Kohara phage 328 in pAMC162. The diagram includes information derived from work discussed in the text (the *cstC* start codon, the site of *kan* cassette insertion in AMS349 [Table 1], the Mu dX fusion joint, and the *cstC* translational stop codon). Numbering is in relation to the *cstC* sequence deposited in GenBank (accession no. U90416); the *Pst*I site is 127 nt upstream of the first nucleotide of this sequence.

spanning nt 418 to 1635 (Fig. 1) exhibited a strong DNA homology to *E. coli N*-α-acetylornithine-δ-aminotransferase, the product of the *argD* gene (map position, 75.1 min). The derived amino acid sequence of the *cstC* ORF revealed a protein of 406 amino acids which, when shifted three residues relative to ArgD, exhibited ca. 60% identity and 91% similarity to the latter (Fig. 2). ArgD belongs to class III of the pyridoxal phosphate (PLP)-dependent aminotransferases; its putative cofactor binding site occurs at Lys^{255} (8). CstC also contains a lysine at this position in the homology alignment (Fig. 2).

Phage Mu dX had inserted in strain AMS35 (and AMS96) near the 3′ end of the *cstC* gene (Fig. 1). As this may not have generated a complete loss-of-function mutation, an additional mutation was constructed by inserting a kanamycin (*kan*) cassette just after Ala^{96} in the CstC polypeptide (Fig. 2), as described previously (6); the Ala^{96} region is vital for the function of amino acid aminotransferases, being involved in subunit dimerization, and thus active-site formation, as well as PLP binding (5). The resulting strain, AMS349, exhibited a phenotype similar to that of AMS96. We thus assume that both AMS35 and AMS349 are loss-of-function mutants.

As opposed to the role for CstC in amino acid catabolism as suggested by the above experiments, ArgD is involved in arginine biosynthesis. However, many closely related enzymes carry out similar biochemical reactions but with equilibria favoring opposite directions, and given the phenotype of the *cstC* mutants, we hypothesize that CstC may be a counterpart of ArgD in a catabolic pathway for amino acids. Indeed, the *E. coli* genome sequence in the *cstC* region (1), as well as biochemical studies presented in an accompanying report (20) indicate that *cstC* (*astC* [20]) is the first gene in a five-gene operon (*astCADBE*). This operon encodes the ammonium-producing arginine succinyltransferase (AST) pathway, which probably catabolizes arginine and other amino acids.

The *cstC* upstream region contains several regulatory sequences. Computer analyses revealed several readily recognizable consensus sequences upstream of the *cstC* ORF (Fig. 3): two each for cyclic AMP-cyclic AMP receptor protein complex (cAMP-CRP) and NR_I binding sites, one for an integration host factor binding site, and consensus sequences for three promoters, σ^{70}, σ^S, and σ^{54}. The putative carbon and ammonium starvation regulatory sites overlap, with the σ^S promoter residing within the σ^{54} promoter and the proximal cAMP-CRP site (nt 107 to 123) located within the two NR_I sites.

We used induction of the *cst-lacZ* fusion in appropriate mutant backgrounds to assess the roles of σ^S and σ^{54} in *cstC* expression. Under ammonium-sufficient growth conditions (i.e., with NH_4^+ as nitrogen source), *cstC* was positively regulated by σ^S, as its expression decreased about twofold in an *rpoS* strain (AMS352) (Table 2), but its expression was not negatively affected in an *rpoN* strain (AMS351). If anything, the presence of σ^{54} in the cells attenuated expression: β-galactosidase production was moderately but reproducibly lower in AMS351. Replacing NH_4^+ with one of several amino acids as nitrogen source (Table 2), thereby generating ammoni-

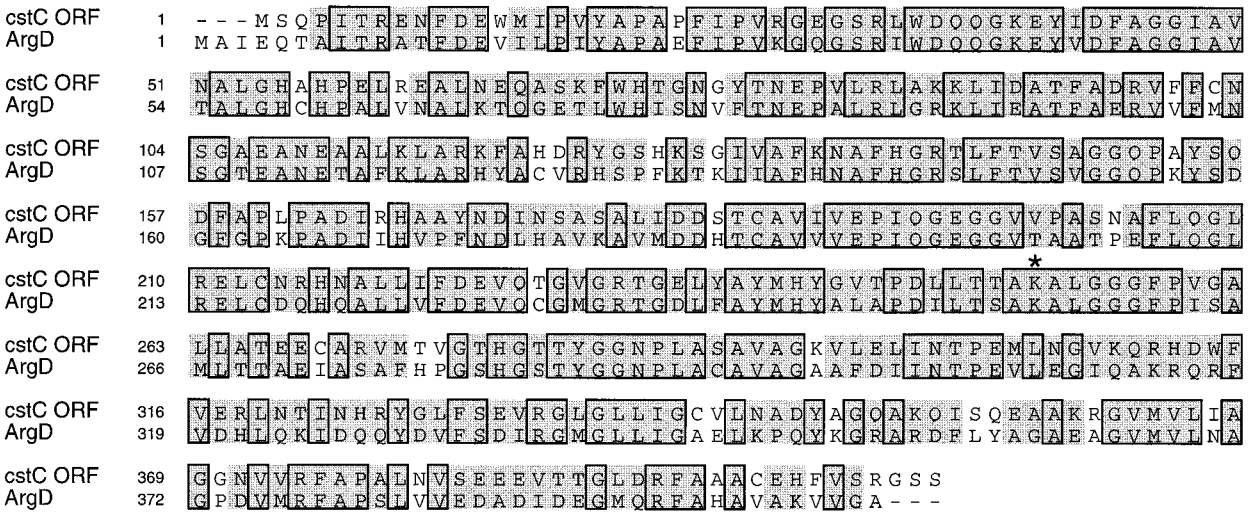

FIG. 2. FASTA (16) amino acid sequence alignment of the putative *cstC* ORF with ArgD. Boxes indicate identical residues; shaded residues indicate conservative substitutions. Note that relative to ArgD, the *cstC* ORF is displaced three residues to the right. The asterisk over the lysines at positions 252 and 255 of the *cstC* ORF and ArgD, respectively, denotes the putative PLP binding site.

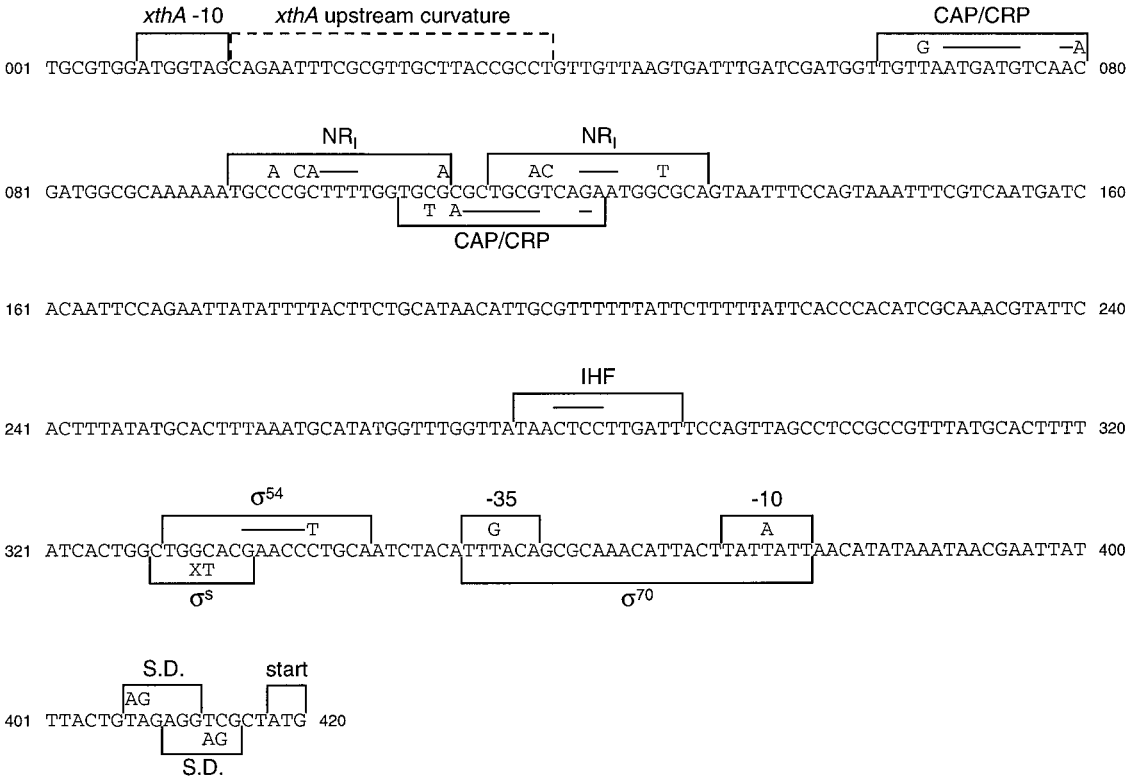

FIG. 3. Sequence of the upstream *cstC* regulatory region from nt 1 of the deposited sequence to the putative start codon. Letters above or below indicate deviations from the consensus sequences, under- or overscores can be any nucleotide, and X denotes where a base should be deleted to obtain the consensus sequence. Abbreviations for the putative sites: CAP/CRP, cAMP-CRP consensus sequence (4); IHF, integration host factor (7); NR_I, nitrogen regulatory protein binding site (11); RpoN, σ^{54} promoter region (17); RpoS, −10 region of the σ^S promoter region (24); −10 and −35, Eσ^{70} promoter sequences (18); S.D., Shine-Dalgarno sequence (22).

um-limited conditions, induced *cstC* expression, but the roles of the two sigma factors were reversed, with σ^{54} acting as the positive regulator and σ^S attenuating expression. Thus, strain AMS351 showed a 3-fold decrease whereas AMS352 showed a 2.5-fold increase in β-galactosidase production under these conditions (Table 2). Qualitatively similar results were obtained during total ammonium starvation (data not shown).

As amino acids are a valuable cellular resource, especially under starvation conditions, a pathway like the AST pathway must be carefully regulated, and the complex regulatory region upstream of the *cstC* gene shows that this is indeed the case. Primer extension start site analysis showed that both σ^{54} and σ^S promoters are used in *cstC* transcription (6a), and the fusion studies discussed above show that when one of these two promoters becomes dominant under a given condition, the other assumes an attenuating role. This competition may be designed to accurately match the expression of the AST pathway to the condition-specific needs of the cell for amino acid catabolism.

TABLE 2. *cstC-lacZ* expression in different backgrounds during ammonium-sufficient and ammonium-limited conditions

Condition	Strain background	β-Galactosidase activity[a]
Ammonium sufficient[b]	AMS96 (wild type)	255
	AMS352 (*rpoS*)	150
	AMS351 (*rpoN*)	300
Ammonium limited[c]	AMS96 (wild type)	1,100
	AMS352 (*rpoS*)	2,800
	AMS351 (*rpoN*)	366

[a] In Miller units, with chlorophenol red–β-D-galactopyranoside as the substrate (10).

[b] Glucose-M9 medium with NH_4Cl as ammonium source.

[c] Glucose-M9 medium minus NH_4Cl with L-arginine, L-lysine, L-ornithine, or *N*-α-acetyl-L-ornithine as ammonium source.

We thank R. Eakins and L. Kozar for help with sequencing and computer analyses, respectively, B. Cormack and K. Wilson for advice, L. Reitzer for providing bacterial strains, and Sara White for expert secretarial help.

This work was supported by grants from the National Science Foundation (ECE86-13227 and DCB-9207101), National Institutes of Health (GM 42159), U.S. EPA (R823390), and Western Region Hazardous Substance Research Center (to A.M.), and ALCOA (to C.D.F.).

REFERENCES

1. **Blattner, F. R., G. Plunkett III, C. A. Bloch, N. T. Perna, V. Burland, M. Riley, J. Collado-Vides, J. D. Glasner, C. K. Rode, G. F. Mayhew, J. Gregor, N. W. Davis, H. A. Kirkpatrick, M. A. Goeden, D. J. Rose, B. Mau, and Y. Shao.** 1997. The completed genome sequence of *Escherichia coli* K-12. Science **277:**1453–1474.
2. **Blum, P. H., S. B. Jovanovich, M. P. McCann, J. E. Schultz, S. A. Lesley, R. R. Burgess, and A. Matin.** 1990. Cloning and in vivo and in vitro regulation of cyclic AMP-dependent carbon starvation genes from *Escherichia coli*. J. Bacteriol. **172:**3813–3820.
3. **Bockman, A. T., C. A. Reeve, and A. Matin.** 1986. Stabilization of glucose-starved *Escherichia coli* K-12 and *Salmonella typhimurium* LT2 by peptidase-deficient mutants. J. Gen. Microbiol. **132:**231–235.

4. **Busby, S. J. W.** 1986. Positive regulation in gene expression. Symp. Soc. Gen. Microbiol. **39:**51–77.
5. **Danishefsky, A. T., J. J. Onnufer, G. A. Petsko, and D. Ringe.** 1991. Activity and structure of the active-site mutants R386Y and R386F of *Escherichia coli* aspartate aminotransferase. Biochemistry **30:**1980–1985.
6. **Fraley, C. D.** 1998. Ph.D. dissertation. Stanford University, Stanford, Calif.
6a. **Fraley, C. D., and A. Matin.** Unpublished data.
7. **Friedman, D. I.** 1988. Integration host factor: a protein for all reasons. Cell **55:**545–554.
8. **Heimberg, H., A. Boyen, M. Crabeel, and N. Glansdorff.** 1990. *Escherichia coli* and *Saccharomyces cerevisiae* acetylornithine aminotransferases: evolutionary relationship with ornithine aminotransferases. Gene **90:**69–78.
9. **Kim, J. H., E. Auger, R. Soly, and A. Matin.** 1993. *E. coli* starvation genes: cloning and analysis of *pexA*, abstr. I-52. *In* Abstracts of the 93rd General Meeting of the American Society for Microbiology 1993. American Society for Microbiology, Washington, D.C.
10. **Lomovskaya, O. L., J. P. Kidwell, and A. Matin.** 1994. Characterization of the σ^{38}-dependent expression of a core *Escherichia coli* gene, *pexB*. J. Bacteriol. **176:**3928–3935.
11. **Magasanik, B.** 1996. Regulation of nitrogen utilization, p. 1344–1356. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed., vol. 1. ASM Press, Washington, D.C.
12. **Matin, A., E. A. Auger, P. H. Blum, and J. E. Schultz.** 1989. Genetic basis of starvation survival in nondifferentiating bacteria. Annu. Rev. Microbiol. **43:** 293–316.
13. **Matin, A.** 1996. Role of alternate sigma factors in starvation protein synthesis—novel mechanisms of catabolite repression, p. 494–505. *In* 14th Forum in Microbiology.
14. **Matin, A., M. Baetens, S. Pandza, C.-H. Park, and S. Waggoner.** Survival strategies in the stationary phase. *In* E. Rosenberg (ed.), Microbial ecology and infectious disease, in press. American Society for Microbiology, Washington, D.C.
15. **McCann, M. P., J. P. Kidwell, and A. Matin.** 1991. The putative σ factor KatF has a central role in development of starvation-mediated general resistance in *Escherichia coli*. J. Bacteriol. **173:**4188–4194.
16. **Pearson, W. R., and D. J. Lipman.** 1988. Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA **85:**2444–2448.
17. **Reitzer, L. J., and B. Magasanik.** 1985. Expression of *glnA* in *Escherichia coli* is regulated at tandem promoters. Proc. Natl. Acad. Sci. USA **82:**1979–1983.
18. **Reznikoff, W. S., and W. R. McClure.** 1986. *E. coli* promoters, p. 1–33. *In* W. Reznikoff and L. Gold (ed.), Maximizing gene expression. Butterworth Publishers, Stoneham, Mass.
19. **Saporito, S. M., B. J. Smith-White, and R. P. Cunningham.** 1988. Nucleotide sequence of the *xth* gene of *Escherichia coli* K-12. J. Bacteriol. **170:**4542–4547.
20. **Schneider, B. L., A. K. Kiupakis, and L. J. Reitzer.** 1998. Arginine catabolism and the arginine succinyltransferase pathway in *Escherichia coli*. J. Bacteriol. **180:**4278–4286.
21. **Schultz, J. E., G. I. Latter, and A. Matin.** 1988. Differential regulation by cyclic AMP of starvation protein synthesis in *Escherichia coli*. J. Bacteriol. **170:**3903–3909.
22. **Stormo, G. D.** 1986. Translation initiation, p. 195–224. *In* W. Reznikoff and L. Gold (ed.), Maximizing gene expression. Butterworth Publishers, Stoneham, Mass.
23. **Ueno-Nishio, S., K. C. Backman, and B. Magasanik.** 1983. Regulation at the *glnL*-operator-promoter of the complex *glnAG* operon of *Escherichia coli*. J. Bacteriol. **153:**1247–1251.
24. **von Ossowski, I., M. R. Mulvey, P. A. Leco, A. Borys, and P. C. Loewen.** 1991. Nucleotide sequence of *Escherichia coli katE*, which encodes catalase HPII. J. Bacteriol. **173:**514–520.