JOURNAL OF BACTERIOLOGY, Apr. 1993, p. 2150–2153
0021-9193/93/072150-04$02.00/0

Vol. 175, No. 7

NOTES

KatF (σ^S) Synthesis in *Escherichia coli* Is Subject to Posttranscriptional Regulation

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Received 7 October 1992/Accepted 24 November 1992

A transcriptional fusion of *katF* to the *lacZ* gene was expressed at increasingly higher levels throughout the exponential phase, but a translational fusion was expressed at low levels during exponential-phase growth and was induced 160-fold during the transition to stationary phase, implicating a posttranscriptional mechanism in the regulation of KatF synthesis. Mutational analyses suggested that the initiation codon of *katF* is the second ATG in the previously identified open reading frame.

The gene *katF* in *Escherichia coli* was initially identified as being required for the synthesis of catalase HPII, encoded by *katE* (9). Subsequently, transcription of a number of genes, including *xthA* (16), *bolA* (2, 7), *appCBA* (22), *mcbA* (2), *otsBA* (4), and genes encoding more than 30 carbon starvation proteins (6, 10) has been shown to be affected by mutations in *katF*. The common feature of all of these genes is that they are expressed as the cells either enter the stationary phase, are transferred from enriched medium to minimal medium, or are subjected to starvation conditions. The implication is that the product of *katF* is controlling a regulon that is turned on in response to nutrient deprivation to help the cell survive under these slow-growth or nongrowth conditions.

A similar gene has been identified in *Salmonella typhimurium* and found to be essential for the expression of virulence genes needed for survival in macrophages (3). In addition, *katF* has been identified in *Shigella flexneri*, in which it is required for development of acid resistance, which allows the organism to survive passage through the gut (20). A *katF*-containing mutant of *S. flexneri* is far less acid resistant and far less virulent than the wild-type strain. Related work has shown that *katF* mutants of *E. coli* are also more sensitive to acid (20) and that *katF* expression may be affected by the internal pH of the cell (18).

The predicted sequence of KatF is very similar to the sequences of σ subunits from bacterial RNA polymerases (13), suggesting a role for the protein which has yet to be demonstrated. Indeed, there are several aspects of KatF physiology that remain unclear. It has been suggested that KatF acts in concert with other factors unique to each system that it controls, so that *katE* expression is turned on as cells enter the stationary phase (8, 14), whereas *xthA* is turned on during the exponential growth phase and apparently turned off in the stationary phase (16). The fact that maximal induction of *katF* transcription does not necessarily lead to full induction of *katE* expression (14) can also be explained in terms of a posttranscriptional control mechanism. This communication identifies the start site of translation and reveals that posttranscriptional control has a role in KatF synthesis.

The published sequence of *katF* (13) identified an open reading frame with a second ATG codon at amino acid 13 which was preceded by a more favorable ribosome-binding site than that preceding the first ATG codon. Thus, it was necessary to determine the actual translation start site. Normally, N-terminal analysis of a gel-purified protein would be used for such a determination. Unfortunately, KatF protein purified after expression from a phage T7 promoter was found to be blocked at its amino terminus, interfering with sequence analysis. Consequently, an alternative method with synthetic promoter segments was used.

Short segments of DNA corresponding to nucleotides −89 to +93 relative to the open reading frame of *katF* (Fig. 1) with protruding *Eco*RI and *Bam*HI ends were constructed from oligonucleotides synthesized on Applied Biosystems 380A and PCR-Mate DNA synthesizers in lengths of 40 to 60 nucleotides. Following phosphorylation by polynucleotide kinase at 37°C for 1 h (17), the fragments were annealed, joined with polynucleotide ligase at 20°C for 16 h, and separated on 2% low-melting-point agarose gels. The gel segment containing the fragment of appropriate size was melted, mixed with plasmid pSK+414 (constructed by inserting the 2.0-kb *Bam*HI-*Sac*I fragment containing the 5′ end of the *lacZ* gene from pRS414 into the M13 Bluescript pSK+ [Stratagene Cloning Systems]) that had been cut with *Eco*RI and *Bam*HI, incubated with polynucleotide ligase at 20°C for 16 h, and used to transform *E. coli* NM522. Selected clones were characterized by sequence analysis. Subsequently, the approximately 2-kb *Eco*RI-*Sac*I fragment containing the synthetic fragment fused to the 5′ end of the *lacZ* gene was inserted in the 8.8-kb *Eco*RI-*Sac*I fragment of pRS414 (20). This construction creates a translational fusion in which the first 31 codons of the *katF* open reading frame are fused to the ninth codon of *lacZ*.

Plasmid pFP20 contained an unmodified *katF* sequence with ATG codons at positions +1 and +37 of the open reading frame (Table 1). Plasmid pFP44 was constructed with an A to C change at +37 (A37C), which resulted in the ATG at +37 being changed to CTG, and plasmid pFP48 was constructed with an A to C change at +1 (A1C), resulting in the ATG at +1 being changed to CTG. The change to a CUG

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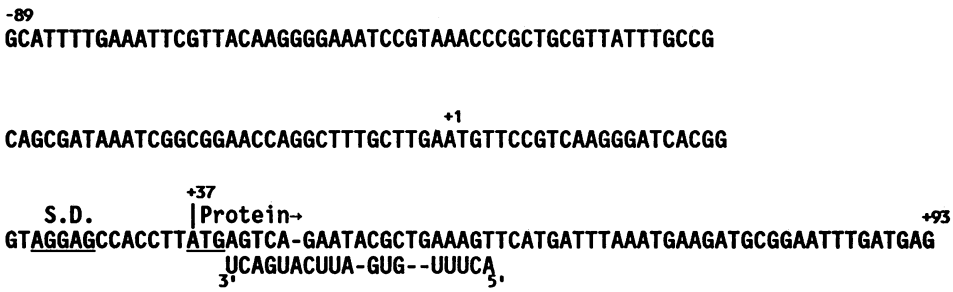

FIG. 1. Sequence of the region of *katF* overlapping the 5′ end of the open reading frame from −89 to +93. The first nucleotide of the open reading frame (13) is indicated by +1. A potential ribosome-binding site (AGGAG) is underlined and indicated above the sequence by S.D. The initiation codon at +37 is indicated above the sequence by |Protein→. The potential anti-downstream box, with the bases 1468 to 1485 of the 16S rRNA arranged to exhibit their complementarity with the *katF* sequence, is shown as a DNA-RNA hybrid.

codon was chosen because CUG has been shown to be only 3% as efficient as AUG in initiating translation (1). The level of expression of β-galactosidase (Miller units [12a]) from pFP48 was the same as the level of expression from pFP20 (Table 2). However, no β-galactosidase was produced from pFP44, in which the ATG at +37 had been changed to CTG, suggesting that this ATG is the actual initiation codon. The low level of expression from these synthetic constructs was due, in part, to the presence of only a low-level promoter in the synthetic sequence (unpublished data) and to the requirement for downstream RNA sequence for optimal translation. For example, shortening the *katF* sequence to position +45, as in plasmid pFP5, resulted in elimination of expression from the translational fusion (Table 2). The positive role for RNA downstream of the initiation codon can be explained by the observation (Fig. 1) that residues 40 to 59 are significantly complementary to a segment of 16S rRNA that has been postulated to be involved in ribosome binding to mRNA (21). This is in contrast to the negative role played by RNA further downstream, which has been postulated to repress translation initiation in the *rpoH* gene (5, 15) and the *katF* gene (11).

The possibility that a posttranscriptional mechanism might be involved in modulating KatF synthesis and the expression of its target genes required investigation. A *katF*::*lacZ* translational fusion, pFP2, was constructed by inserting the 0.9-kb *Rsa*I fragment from pMF2 (14), containing *katF* sequence extending from −350 to +526, into the *Sma*I site of pRS414. This resulted in the first 163 amino acids (starting from the ATG at +37) being fused to the amino terminus of β-galactosidase. The levels of β-galactosidase activity in pFP2-transformed cells grown in LB medium are shown in Fig. 2A. For comparison, the activities resulting from expression of the transcriptional fusions pRSkatF5, which contains the *katF* sequence from −500 to +65 relative to the open reading frame, and pFP53, which contains the same −350 to +526 fragment as in pFP2, are shown (Table 3). Expression from the fusion plasmid pFP50 containing only the *katF* sequence from −292 to +65 was the same as from pRSkatF5, confirming that the region upstream of −292 in pRSkatF5 is not required for expression. Inclusion of the region between +65 and +526, as in pFP53, caused a 50% reduction in transcriptional expression. The significant difference between the patterns of expression from pRSkatF5 and pFP2 is the time that expression is turned on. Expression of the transcriptional fusion increased throughout the exponential phase, reaching a maximum in the stationary phase. Expression of the translational fusion was low during the exponential phase and increased more abruptly as cells

TABLE 1. *E. coli* strain, plasmids, and bacteriophages used

Strain, plasmid, or phage	Relevant characteristics	Source or reference
E. coli NM522	*recA supE* Δ(*lac-proAB*) *hsd-5* (F′ *proAB lacI*^{q}*15*)	12
Plasmids		
pRS414		19
pRS415		19
pSK+		Stratagene Cloning Systems
pMF5		13
pSK+414	2.0-kb *Eco*RI-*Sac*I fragment from pRS414 into *Eco*RI-*Sac*I-cut pSK+	
pRSkatF5		14
pFP2	0.9-kb *Rsa*I fragment from pMF2 (−350/+526) into *Sma*I-cut pRS414	
pFP8	134-bp segment (−89/+45 in Fig. 1) in pSK+414	
pFP5	2.0-kb *Eco*RI-*Sac*I fragment from pFP8 in *Eco*RI-*Sac*I-cut pRS414	
pFP18	182-bp segment (−89/+93 in Fig. 1) in pSK+414	
pFP20	2.0-kb *Eco*RI-*Sac*I fragment from pFP18 in *Eco*RI-*Sac*I-cut pRS414	
pFP42	182-bp segment (−89/+93 in Fig. 1, A37C) in pSK+414	
pFP44	2.0-kb *Eco*RI-*Sac*I fragment from pFP42 in *Eco*RI-*Sac*I-cut pRS414	
pFP46	182-bp segment (−89/+93 in Fig. 1, A1C) in pSK+414	
pFP48	2.0-kb *Eco*RI-*Sac*I fragment from pFP46 in *Eco*RI-*Sac*I-cut pRS414	
pFP50	0.4-kb *Eco*RI-*Dra*I fragment from pMF5 in *Eco*RI-*Sma*I-cut pRS415	
pFP53	0.9-kb *Eco*RI-*Bam*HI fragment from pFP2 in *Eco*RI-*Bam*HI-cut pRS415	
Phages		
λRS45		19
λFP2	λRS45 × pFP2	
λRSkatF5	λRS45 × pRSkatF5	
λFP53	λRS45 × pFP53	

TABLE 2. Translational expression from various synthetic *katF* promoters on multicopy plasmids

Promoter region (mutation)	Plasmid	β-Galactosidase activity (Miller units) in stationary phase[a]
−89/+93	pFP20	4 (4.84)
−89/+93 (A1C)	pFP48	4 (3.28)
−89/+93 (A37C)	pFP44	<0.5 (4.31)
−89/+45	pFP5	<0.5 (3.60)

[a] The values in parentheses are the A_{600} readings of cell density.

made the transition to stationary phase. Clearly, there is posttranscriptional control of KatF synthesis; it is induced coincident with the induction of *katE* transcription (14) and catalase HPII appearance (8).

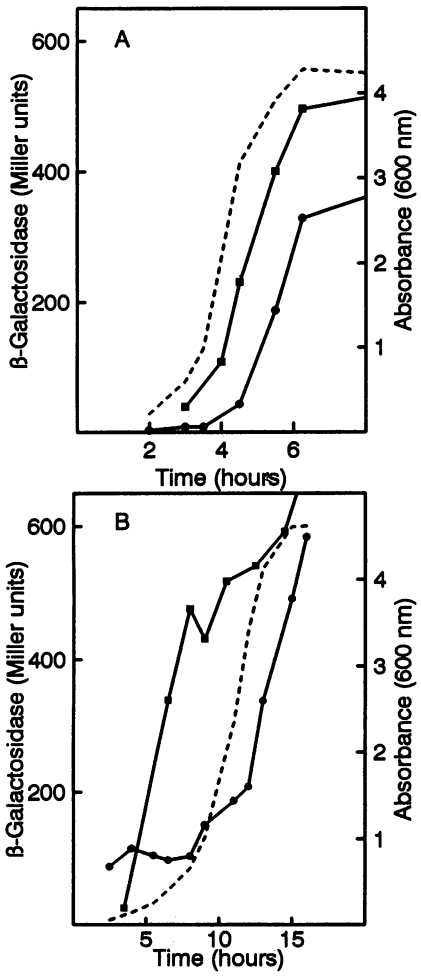

FIG. 2. β-Galactosidase activity from *lacZ* under the control of the *katF* promoter fused for transcriptional expression (■) and translational expression (●). The values for transcriptional expression were all divided by six for this figure so that the same scale could be used. (A) Cultures of NM522(pRSkatF5) (■) and NM522(pFP2) (●) were grown in LB medium. The dashed line represents the A_{600} as a function of time. Samples were removed and assayed for β-galactosidase levels at various times. (B) Cultures of NM522(pFP50) (■) and NM522(pFP2) (●) were grown in LB supplemented with 20 mM sodium benzoate.

TABLE 3. Transcriptional and translational expression of *katF* from multicopy plasmids and single-copy λ bacteriophage lysogens determined in exponential-phase and stationary-phase cultures

Fusion type and plasmid or phage	β-Galactosidase activity (Miller units)[a]	
	Exponential phase	Stationary phase
Transcriptional		
pRSkatF5	154 (0.23)	2,992 (3.96)
pFP50	335 (0.55)	2,981 (5.51)
pFP53	241 (0.25)	1,771 (4.61)
λRSkatF5	10 (0.16)	155 (4.10)
λFP53	16 (0.47)	77 (4.56)
Translational		
pFP2	3 (0.22)	523 (4.02)
λFP2	<0.5 (0.32)	18 (4.19)

[a] The numbers in parentheses are the A_{600} readings of cell density.

A second experiment, shown in Fig. 2B, confirmed this apparent close relationship between *katF* translation and *katE* transcription. It was reported (14) that aromatic acids in LB medium induced *katF* transcription fully but induced *katE* transcription to only a third of its maximum level. As shown in Fig. 2B, the presence of benzoate caused a partial turn-on of *katF* translation to only 25% of maximum despite the full induction of transcription. In other words, the pattern of *katF* translation was very similar to the pattern of *katE* transcription. Posttranscriptional regulation of KatF synthesis might involve differential mRNA stability, mRNA interaction with ribosomes, translation initiation, or ribosome movement. Further work is required to distinguish among these possibilities.

The translational fusion in pFP2 and the transcriptional fusions in pRSkatF5 and pFP53 were transferred into λRS45 (19) by homologous recombination, and the resulting phage were used to generate single-copy lysogens. Expression of β-galactosidase in the lysogens was approximately 30-fold lower in both exponential- and stationary-phase cultures (Table 3). However, the patterns of expression during growth were unchanged from those shown in Fig. 2 (data not shown), indicating that the presence of multiple gene copies did not affect expression.

This work was supported by grants OGP0009600 and ICRO121180 (to P.C.L.) and a postgraduate scholarship (to I.V.) from the Natural Sciences and Engineering Research Council.

We thank H. G. Khorana and U. L. RajBhandary for accommodating P.C.L. in their laboratories while he was on sabbatical leave and for many helpful discussions.

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