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The Putative σ Factor KatF Is Regulated Posttranscriptionally during Carbon Starvation

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Transcriptional and translational *'lacZ* reporter fusions were constructed to the *katF* gene, which encodes a putative σ factor centrally involved in starvation-mediated general resistance in *Escherichia coli*. Transcription of *katF* was found to increase ca. twofold after carbon starvation in minimal medium. The protein fusion containing the longest fragment of *katF* induced ca. eightfold under the same conditions, whereas fusions to shorter segments showed only a twofold increase in expression. The protein fusion was expressed at higher levels in a strain containing a *katF*::Tn*10* mutation, indicating *katF* autoregulation. The posttranscriptional regulation of *katF* by starvation did not require a component of the spent minimal medium. *katF* was also posttranscriptionally regulated during entry into late log phase in complex medium. This induction was coincident with an increase in *katE* transcription, suggesting that the cellular concentration of KatF directly followed the induction of the *katF* protein fusion.

When *Escherichia coli* is starved for carbon in a minimal medium, it induces over 50 proteins in a temporally regulated fashion (2, 13–15, 27). The induction of at least 32 of these proteins requires the putative σ factor KatF, since a strain containing a *katF*::Tn*10* mutation fails to induce these proteins (9, 15). The KatF-regulated starvation proteins include several members of the Pex class (15), whose induction is thought to be responsible for the generalized resistant state that *E. coli* acquires upon starvation (2, 4–6, 13, 27); indeed, the *katF* mutant is impaired in development of starvation-mediated general resistance (9, 15).

Although, as noted above, some 32 of the KatF-dependent starvation proteins are markedly induced upon carbon starvation in a minimal medium—the increased expression of at least one of which (PexB) involves an increase in *pexB* transcription (8)—there is little (21) or no (9) induction of *katF* transcription under these conditions. This has been shown by using *'lacZ* transcriptional fusions either to the *katF* gene on a multicopy plasmid (21) or to the chromosomal copy of *katF* (9). This suggests that *katF* is regulated at a posttranscriptional level.

Accordingly, we tested a series of *'lacZ* protein and operon fusions at several locations in the *katF* open reading frame. We report here that although *katF* is regulated at the transcriptional level, the more important regulation is posttranscriptional during growth and carbon starvation in minimal and complex media.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth media. The *E. coli* strains and plasmids used in this study are listed in Table 1. LB and M9 minimal media were prepared as described previously (18). When specified, the media were supplemented with ampicillin (50 μg/ml), kanamycin (25 μg/ml), and/or 5-bromo-4-chloro-3-indoyl-β-D-galactopyranoside (X-Gal, 50 μg/ml).

Molecular biology protocols and methods. Restriction digests, ligations, and DNA purifications were performed as described before (12). T4 DNA ligase and restriction enzymes were purchased from New England Biolabs. Plasmids were introduced into cells by electroporation (17). Dideoxy sequencing was performed using the Sequenase Version 2.0 DNA sequencing kit (United States Biochemical) according to the manufacturer's protocol. β-Galactosidase activity was determined by the method of Clark and Switzer (1) and is expressed as nanomoles of *o*-nitrophenyl-β-D-galactoside hydrolyzed per minute per A_{660} unit.

Polymerase chain reaction (PCR) was used to generate different-length segments of the *katF* gene flanked by unique restriction sites. Amplitaq DNA polymerase (Perkin Elmer Cetus) was used as per the manufacturer's directions. Reaction conditions involved denaturation at 94°C for 45 s, annealing at 50°C for 1 min, and extension at 72°C for 1 min. Four PCR primers were used: 1, 5′-GCGGGATCCCGCATTAAAGCTTATCGAT-3′; 2, 5′-GCGGAATTCAACAGTGGTGAATAACCA-3′; 3, 5′-GCGGAATTCATAATCGCCCGTTCAATC-3′; and 4, 5′-GCGGAATTCGTGGTATCTTCCGGACCG-3′. Primers were synthesized using an Applied Biosystems model 394 DNA/RNA synthesizer. Primer 1 is complementary to sequences in pAT153 (30) immediately upstream of and including the *Cla*I site into which the DNA fragment containing the *katF* gene has been cloned (Fig. 1) (19, 20). Primers 2 through 4 are complementary to sequences internal to the *katF* open reading frame from bases +231 to +248, +495 to +515, and +768 to +786, relative to the first potential translational start codon (19), respectively. Recently, Loewen et al. (11) reported that the second ATG codon at +37 is more likely to be the translation initiation site. However, they retained the original numbering scheme (11), prompting us to follow it here. The underlined segment of primer 1 is a *Bam*HI restriction site. The underlined segments of primers 2 through 4 contain *Eco*RI sites. Twenty picomoles of each primer was used. Primer 1 was used in conjunction with primer 2, 3, or 4 in PCRs with 1 μg of pMMkatF3 (the plasmid containing the *katF* gene [20]) as the

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

Strain or phage	Relevant characteristics	Source[a] or reference
E. coli		
K-12	Wild type (λ^- F^-)	27
NC1	*katE*::λ*plac*Mu53	25
AMS6	K-12, Δ*lacU169*	27
AMS8	Like AMS6 but Δ*cya-856*	27
AMS150	Like K-12 but *katF*::Tn*10*	15
AMS153	Like AMS6 but λMPM1	This study
AMS154	Like AMS6 but λMPM2	This study
AMS155	Like AMS6 but λMPM3	This study
AMS156	Like AMS6 but λMPM4	This study
AMS157	Like AMS6 but λMPM5	This study
AMS158	Like AMS6 but λMPM6	This study
AMS159	Like AMS6 but *katE*::λ*plac*Mu53	P1(NC1) × AMS6 →Kan^r
AMS164	Like AMS157 but *katF*::Tn*10*	P1(AMS150) × AMS157 →Tc^r
AMS165	Like AMS158 but *katF*::Tn*10*	P1(AMS150) × AMS158 →Tc^r
AMS166	Like AMS8 but λMPM5	This study
AMS167	Like AMS8 but λMPM6	This study
AMS168	Like AMS166 but *katF*::Tn*10*	P1(AMS150) × AMS166 →Tc^r
Phages		
P1 *vir*		Our laboratory strain
λRS45		28
λMPM1	Like λRS45 but with *'lacZ* operon fusion to base +248 of *katF*	This study
λMPM2	Like λRS45 but with *'lacZ* protein fusion to base +248 of *katF*	This study
λMPM3	Like λRS45 but with *'lacZ* operon fusion to base +515 of *katF*	This study
λMPM4	Like λRS45 but with *'lacZ* protein fusion to base +515 of *katF*	This study
λMPM5	Like λRS45 but with *'lacZ* operon fusion to base +786 of *katF*	This study
λMPM6	Like λRS45 but with *'lacZ* protein fusion to base +786 of *katF*	This study

[a] Donor strains for P1 transductions are shown in parentheses. Kan^r, kanamycin resistance; Tc^r, tetracycline resistance.

target (Fig. 1). Typically, five to seven rounds of amplification were used, depending upon the yield of the product.

The sequence of the promoter regions of the PCR clones was determined with an Applied Biosystems model 373A automated DNA sequencer. Plasmid DNA containing the various fusions was purified by passage through Qiagen columns as per the manufacturer's instructions after alkaline lysis. Two sequencing primers were used: A, 5'-AGTGAT CGAAACCTTTGGCG-3' (−364 to −345, forward primer); and B, 5'-AAGGTGGCTCCTACCCGTGA-3' (+36 to +17, reverse primer). The results showed that the PCR procedure did not result in any mutations.

Starvation protocols. Starvation experiments were typically performed by first subculturing an overnight culture into fresh, prewarmed medium. This culture was grown at 37°C with shaking at 150 rpm for at least three generations and was then subcultured 1:10 into prewarmed experimental medium. This point is represented as time zero in the figures in this paper.

Carbon starvation in minimal medium was attained using the above procedure with M9 medium supplemented with 0.025% (wt/vol) glucose and 25 μg of kanamycin per ml. The onset of starvation was determined by the cessation of growth, as measured by the A_{660}, which has been shown to be coincident with glucose exhaustion under these conditions (2). Alternatively, carbon starvation was induced by harvesting cultures growing exponentially in M9 medium containing 0.4% glucose by centrifugation for 5 min at 5,000 × *g* and 4°C. The cultures were washed twice with 2 volumes of sterile, ice-cold M9 medium with no carbon source. Cultures were then resuspended in M9 medium devoid of a carbon source. At this point, the A_{660} of the culture was ca. 0.25. These cultures were incubated with shaking as described above. As a control for the effects of centrifugation and washing, cells were also resuspended in the supernatant saved from the harvesting step and held at 37°C. The washing was found to have no effect on the growth rate of the control culture (data not shown). Two-dimensional polyacrylamide gel electrophoresis analysis of de novo protein synthesis immediately before and after the washing also showed no changes in the pattern of protein synthesis in the controls (data not shown).

Starvation in complex medium was attained by allowing the cultures to proceed into stationary phase.

RESULTS

Construction of *'lacZ* reporter gene fusions to *katF*. Prior to preparing the PCR primers, we sequenced the cloned *katF* gene and upstream region in pMMkatF3 to confirm the sequence of this region as reported by Mulvey and Loewen (19). A number of discrepancies from the published sequence were found (Fig. 2). Resequencing by P. C. Loewen has confirmed these differences (10a).

Single-copy reporter gene fusions to *katF* were constructed using the system of Simons et al. (28). To generate in-frame fusions, segments of the *katF* gene were isolated by PCR as described in Materials and Methods. The primers used in the PCR reaction were constructed so that they contained either a *Bam*HI restriction site (primer 1) or an *Eco*RI restriction site (primers 2 through 4) at their ends (Fig. 1). The resulting PCR products thus contained unique restriction sites at both ends. These sites permitted directional cloning of the three *katF* gene segments into the *'lacZ* fusion vectors pRS550 and pRS577 to construct operon and protein fusions, respectively. The three sets of fusions to *'lacZ* were joined to the *katF* gene at base +248, +515, or +786 relative to the first *katF* ATG (11, 19). The 5' ends of

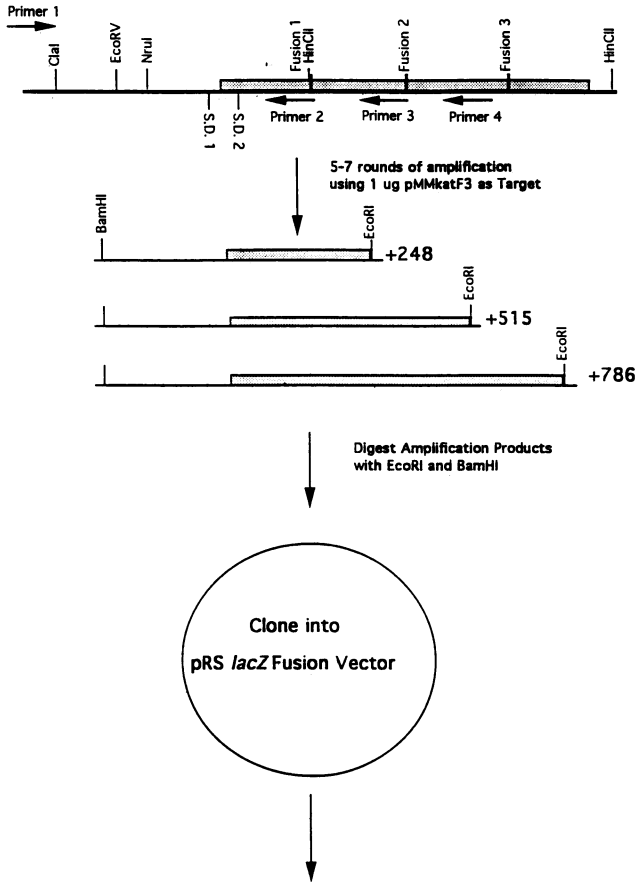

FIG. 1. Construction of lysogens containing *katF* operon and protein fusions. Segments of the *katF* gene were isolated by PCR. Primer 1, which was complementary to vector sequences, was used in combination with primer 2, 3, or 4 to generate gene segments ending at base +248, +515, or +786, respectively. Primers 2, 3, and 4 contained an *Eco*RI site positioned so that the *katF* gene is in frame with the *'lacZ* gene in pRS577 (28). Each of the three segments generated was cloned into both the protein (pRS577) and operon (pRS550) fusion vectors (28). The fusions contained on these plasmids were recombined onto λRS45 to generate phages λMPM1 through λMPM6, which were then lysogenized in strain AMS6. S.D.1 and S.D.2 refer to the putative Shine-Dalgarno sequences (19); see text for further details.

all the fusions were identical, starting just upstream of the *Cla*I site in the vector pAT153 (30). Thus, the fusions contained approximately 1.3 kb of DNA 5′ to the first ATG. It is likely that this region contains all the *katF* promoter elements.

The fusion joints of the plasmid constructs were confirmed by sequence analysis (data not shown). Approximately 100 transformants of each type of fusion were examined on LB agar containing X-Gal. There was no apparent difference in β-galactosidase expression between isolates of each fusion type, as judged by the intensity of the blue color. Three isolates of each type of fusion were then recombined onto λRS45 (28). Lysogens were constructed in AMS6 using these 18 recombinant phages. The kinetics of β-galactosidase expression of each isolate in minimal glucose medium was examined and found to be essentially identical (data not shown). This is consistent with the premise that the PCR process had not introduced mutations which affected the regulation of the *katF* gene segments. One isolate from each group was then chosen at random for further analysis.

As part of the characterization of the *katF* fusions, the sequence of the promoter region was determined to ensure that no errors had been induced during PCR. All of the PCR clones were found to have the same DNA sequences in the promoter region, consistent with the sequence presented in Fig. 2.

Regulation of *katF* reporter fusions in minimal glucose medium. The expression of the *katF* gene during growth and carbon starvation in M9 medium plus glucose was assayed using the six reporter fusions. The expression of the fusions to base +515 is shown in Fig. 3A; fusions to base +248 had the same pattern of expression (data not shown). Both the transcriptional and translational fusions consistently exhibited a twofold induction. This induction began and was complete before growth of the culture stopped.

The reporter fusions to base +786 exhibited a different pattern of expression than did the shorter fusions (Fig. 3B). The operon fusion (AMS157) was induced at the onset of starvation, and the twofold induction it exhibited was complete within the first hour of starvation. The protein fusion to base +786 (AMS158) closely followed the induction pattern of AMS157 for the first hour of starvation; however, unlike the latter, its induction continued for another 3 h, resulting in a more than eightfold increase (Fig. 3B). Thus, it appears that the regulation of *katF* expression is at the transcriptional level in early starvation, followed by further induction controlled by posttranscriptional mechanisms. This induction most likely results in increased cellular levels of KatF during carbon starvation, which is consistent with our previous observation that some 32 KatF-regulated proteins are induced upon glucose exhaustion (15); with the finding that the induction of at least one Pex protein, PexB, involves a KatF-dependent increase in the transcription of its gene under these conditions (8); and with the data presented below.

The β-galactosidase synthesis patterns in strains AMS157 and AMS158 actually reflect reduced levels of expression during growth compared to the shorter fusions rather than increased induction upon starvation, since all the fusions attained approximately the same level of β-galactosidase activity during carbon starvation (Fig. 3A and B).

Starvation by resuspension of washed cells in a medium devoid of a carbon source is physiologically different from starvation resulting from exhaustion of a nutrient because in the latter case, inducers, repressors, etc., accumulated during growth can influence the starvation response. Indeed, components of spent complex medium can induce transcription of the *katF* gene (20, 21, 25, 26). We therefore examined the posttranscriptional regulation of *katF* during starvation by resuspension. Cultures of AMS157 and AMS158 growing exponentially in M9 glucose medium were harvested and washed. Half of each culture was then resuspended back in the original growth medium to serve as a control, while the remainder was resuspended in M9 medium devoid of a carbon source. The starved culture of AMS157 showed no increase in β-galactosidase levels (Fig. 4), indicating that transcription of *katF* was not induced during starvation by resuspension. However, consistent with our findings of the induction of the KatF-regulated starvation proteins also under these conditions (2), expression of the protein fusion (AMS158) increased ca. fourfold. Thus, although less

```
                Primer A
                ---------->
-364 AGTGATCGAAACCTTTGGCGCTTCTGAGGGGGGCAACAAGGGGATTGATATCGCAGGCAGC -304

-303 AAAGACAGCAATTATCGCGACCGCAGATGGCCGCGTTGTTTA-TGCTGGTAACGCGCTGCG -244
                **                          *  *  *
                -293 TCGCGACCTGAGATGGCCG-GTTGTTTAGTGCTGGTAA-TCGTTGTG -250

-243 CGGCTAC-GGTAA-TCTGATTATCATCAAA-CATAATGATGATT-A-CCTGAGT-GCC-TA -190
      * **     *
-249 GGGTGACTGGTAAGTCTGATTATCATGAAATCATAATGATGATTCAGCCTGAGTAGCCTTA -189

-189 CGCCCATAA-CGACACAA-TGCTGGTCCGGGAACAACAAGAAGTTAAGGCGGGGCAAAAAA -131
                                                 *
-188 CGCCCATAACCGACACAAGTGCTGGTCCGGGAACAACAAGAAGTTAAGGCGGGGAAAAAAA -128

-130 TAGCGACCATGGGTAGCACCGGAACCAGTTCAACACGCTTGCATTTTGAAATTCGTTACAA  -70
-127 TAGCGACCATGGGTAGCACCGGAACCA-TTCAACACGC-TGCATTTTGAAATTCGTTACAA  -69

 -69 GGGGAAATCCGTAAACCCGCTGCGTTATTTGCCGCAGCGATAAATCGGCGGAACCAGGCTT   -9
 -68 GGGGAAATCCGTAAACCCGCTGCGTTATTTGCCGCAGCGATAAATCGGCGGAACCAGGC-T   -9
                                Primer B
                <----------
  -8 TTGCTTGAATGTTCCGTCAAGGGATCACGGGTAGGAGCCACCTTATGAGTCAGAATAC     +50
  -8 TTGCTTGAATGTTCCGTCAAGGGATCACGGGTAGGAGCCACCTTATGAGTCAGAATAC     +50
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FIG. 2. Nucleotide sequence of the region upstream of the *katF* open reading frame beginning at −364 bp. The previously published sequence (lower line; beginning at −293 bp [19]) is provided for comparison. Mismatches are indicated by * and gaps by —. The first and second possible start codons (see text) are underlined, and the sequences complementary to sequencing primers A and B are overlined.

marked, an increase in KatF levels also occurred in the absence of any factors accumulated during growth.

Effect of a Δ*cya* mutation on expression of the *katF* reporter fusions. Previous studies (9) have shown that the *cya* gene negatively affects transcription of *katF*. We therefore examined the effect of this gene on the regulation of transcriptional *katF* fusions. Phage λMPM5, containing the operon fusion to base +786 of *katF*, was P1 transduced into the Δ*cya* strain AMS8 to generate strain AMS166. During growth in M9 medium, β-galactosidase production in strain AMS166 was nearly half that in *cya*-proficient strain AMS157 (32 versus 72 U, based on five independent measurements). Additionally, there was no induction of β-galactosidase activity in AMS166 at the onset of glucose starvation (data not shown), whereas in AMS157, expression of the operon fusion increased ca. twofold (Fig. 3A).

This is in contrast to the finding by Lange and Hengge-Aronis that transcription of *katF* was increased in a Δ*cya* strain (9). One explanation for this discrepancy is that the strain used here was *katF*$^+$, whereas they used a *katF* mutant strain. We therefore transduced a *katF*::Tn*10* mutation into strain AMS166 to generate strain AMS168. The double mutant was markedly impaired in growth in minimal glucose medium, with a generation time of 360 min, versus 70 min for the wild type and 130 min for the Δ*cya* strain, which agrees with the previous study (9) with respect to the effect of these mutations on growth rate. But the expression of the *katF* operon fusion in strain AMS168 was identical to that in strain AMS166 (data not shown), indicating that the discrepancy between our results and those of Lange and Hengge-Aronis is not related to the presence or absence of a functional *katF* gene.

If a Δ*cya* background reduces *katF* transcription and abolishes its induction by starvation, as shown above, how

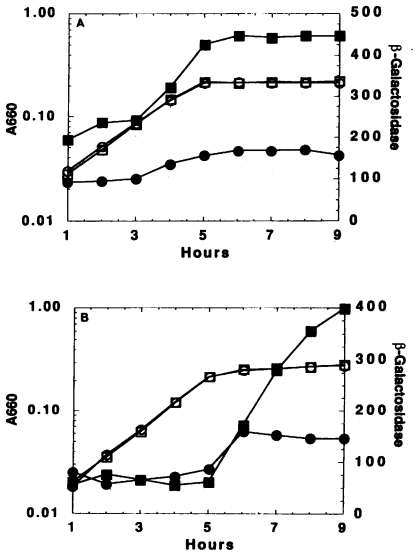

FIG. 3. β-Galactosidase synthesis during growth and glucose starvation in M9 medium containing 0.025% glucose by *'lacZ* operon (○, ●) or protein (□, ■) fusions to *katF* at base +515 (AMS155 and AMS156, respectively) (A) or +786 (AMS157 and AMS158, respectively) (B). Growth (open symbols) was monitored by A_{660} measurements. β-Galactosidase activity (solid symbols) was measured as described in Materials and Methods.

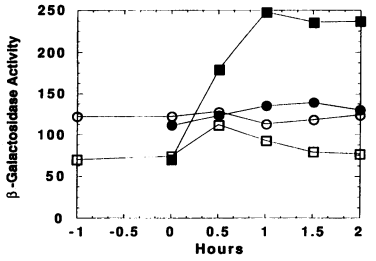

FIG. 4. β-Galactosidase synthesis by *'lacZ* operon (AMS157; ○, ●) or protein (AMS158; □, ■) fusions to *katF* at base +786 during carbon starvation produced by resuspension of exponentially growing cells in glucose-free medium. The experimental culture was resuspended in glucose-free medium at time zero (solid symbols). Control cultures were resuspended in growth medium (open symbols).

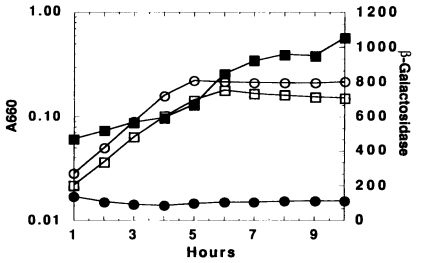

FIG. 5. Growth curves (open symbols) and β-galactosidase synthesis during growth and starvation in M9 medium (0.025% glucose) (solid symbols) by *'lacZ* fusions to base +786 of *katF* in a *katF*::Tn*10* background. ○, ●, AMS164 (*'lacZ* operon fusion); □, ■, AMS165 (*'lacZ* protein fusion).

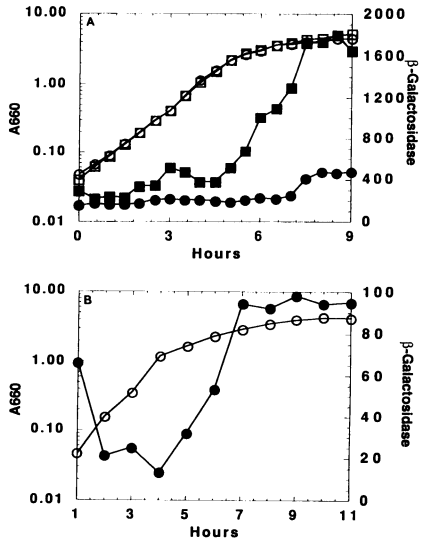

FIG. 6. β-Galactosidase synthesis by *'lacZ* fusion to *katF* or *katE* in complex medium. (A) Cells containing protein (AMS156; □, ■) and operon (AMS155; ○, ●) fusions to base 515 of *katF* were grown in LB medium. Growth of the cultures was monitored by A_{660} measurements (open symbols), and β-galactosidase activity was determined (solid symbols). (B) Expression of *katE* using an operon fusion was determined under the same conditions.

are the KatF-regulated starvation proteins induced during carbon starvation in M9 medium in a Δ*cya* strain, as we have previously reported (27)? When the protein fusion to +786 was examined in a Δ*cya* background (strain AMS167) it exhibited ca. 250 U of β-galactosidase activity during growth as well as starvation. Thus, KatF is most likely present in a Δ*cya* background (primarily because of posttranscriptional regulation), and in this background, changes in KatF levels may not be involved in starvation protein induction after carbon exhaustion. This suggests that starvation produces some other unknown trigger for the induction of these proteins, which is unmasked in this background.

Effect of a *katF*::Tn*10* mutation on expression of the *katF* fusions. In the course of constructing the Δ*cya katF*::Tn*10* double mutant, we noted that expression of the *katF* protein fusion was increased during growth and carbon starvation in this background (data not shown). We therefore examined the possibility that *katF* is autoregulated. A *katF*::Tn*10* mutation was introduced into strains AMS157 and AMS158 by P1 transduction to generate strains AMS164 (operon fusion to base +786) and AMS165 (protein fusion to base +786).

During growth in M9 medium, β-galactosidase synthesis by the operon fusion AMS164 (Fig. 5) was somewhat higher than that found in strain AMS157 (Fig. 3B), but unlike the latter, no increase in expression occurred during carbon starvation. Expression of the protein fusion (AMS165) was markedly increased in this background compared to expression in AMS157, and significant induction occurred at the onset of starvation (Fig. 5): levels were over 11-fold higher during growth and 2.5-fold higher during starvation compared to strain AMS158. This indicates that *katF* either directly or indirectly regulates itself at both the transcriptional and posttranscriptional levels. Autoregulation of *katF* in complex medium has recently been reported (26).

Regulation of *katF* reporter gene fusions in complex medium. Previous studies have shown that the transcription of both *katE* and *katF* is induced as cells enter mid- to late log phase in complex media (10, 21, 24–26). We therefore determined if *katF* was also posttranscriptionally regulated under these conditions. The expression of the *katF* operon fusion to base +515 (AMS155) was increased over 2.5-fold as cells entered the stationary phase (Fig. 6A), in agreement with previous reports. Prior to this increase in *katF* transcription, expression of the protein fusion to base +515 (AMS156) began to increase approximately sixfold. This increase began after the growth rate of the culture had begun to slow down (Fig. 6A). The other operon and protein fusions (+248 and +786) showed identical patterns of regulation under these conditions (data not shown). Loewen et al. also found that when transcriptional and translational fusions with the same fusion joint are studied, *katF* transcription and translation are induced mainly in the stationary phase in complex medium (Fig. 2A and Table 3 in reference 11).

Does the increased expression of the *katF* protein fusion represent an increase in the intracellular KatF level? This question could not be addressed directly because no antibody against KatF is available. However, as an indirect indication of *katF* transcriptional activity in vivo, the induction of a *katE*::*lacZ* operon fusion was examined; the transcription of *katE* is KatF dependent (21). Strain AMS159, containing the single-copy *katE*::*lacZ* operon fusion, was grown in LB medium. Transcription of *katE* began to increase as the culture entered the late log phase and the growth rate began to decrease (Fig. 6B). This increase occurred at approximately the same phase of growth as did the expression of the *katF* protein fusion and before any induction of *katF* transcription (Fig. 6A). An operon fusion to another KatF-regulated gene, *pexB*, showed very similar kinetics of induction (8). These results suggest that the induction of the protein fusions does coincide with increased KatF levels in the cells.

DISCUSSION

The studies reported in this paper were initiated primarily because previous data indicated that some 32 KatF-dependent starvation proteins were induced upon carbon starvation in minimal medium without coincident induction of *katF* transcription. We show here that *katF* expression is in fact regulated at the transcriptional level during starvation under these conditions but that the more important component of this regulation is posttranscriptional. Thus, in this medium, with the fusions to base +786, an only twofold induction of

transcription during starvation was accompanied by an eightfold induction of the protein fusion. A similar situation occurred in the complex medium regardless of the length of the *katF* gene segment contained in the fusions. Furthermore, the activity of KatF, as assayed by transcription of two KatF-dependent genes, closely followed the induction of the *katF* protein fusions in the complex medium (8). In fact, *katE* transcription reached a maximum before the *katF* operon fusion exhibited any induction. Similarly, starvation by resuspension caused a rapid increase in expression of the *katF* protein fusions without increasing that of the *katF* operon fusion; we have previously shown that KatF-dependent proteins are induced under these conditions (2, 15). Taken together, these findings strongly suggest that regulation, primarily at the posttranscriptional level, modulates the cellular KatF concentration to control the expression of the KatF starvation regulon. It is also noteworthy that in minimal medium, the posttranscriptional induction assumes importance after the first hour of starvation (Fig. 3B), which is the time when most KatF-regulated Pex proteins are maximally synthesized (2). We are now attempting to raise an antibody to KatF so as to directly measure cellular changes in KatF concentration at the onset of starvation, as we have done previously with σ^{32} (4).

The regulation of *katF* transcription is complex. Cyclic AMP appears to play a role in stimulating the transcription of *katF*. This observation was initially surprising in light of the ability of Δ*cya* strains to induce KatF-regulated starvation proteins (27). However, the kinetics of expression of the protein fusions suggest that the posttranscriptional regulatory mechanisms can compensate for the lack of increased *katF* transcription in the absence of cyclic AMP. This type of multiple regulation has been observed in other stress response systems such as the heat shock regulon (22).

The mechanism by which *katF* expression is regulated at the posttranscriptional level is presently unclear. It appears that during growth in minimal glucose medium, expression of *katF* is repressed and that this repression requires sequences between bases +515 and +786. These sequences are not required for maximal expression during carbon starvation in this medium, since even the fusion to base +248 reaches the same level of expression as the fusion to base +786 during starvation. This repression may be the result of an interaction between bases downstream of +515 with those near the translation initiation site, as is predicted by computer projection of *katF* mRNA secondary structure (16a). The predicted secondary structure indicates that bases +551 to +554 could interact with the second ATG, while bases +669 to +673 could interact with the second possible ribosome-binding site. This interaction could exclude ribosomes from translating the message, especially in light of the observation that the first ATG is not used in translation (11).

Such posttranscriptional repression would be analogous to that recently described in the regulation of σ^{32} (7, 23). In this system, the sequences containing the ribosome-binding site and the start codon are thought to interact with downstream sequences within the *rpoH* coding region. The resulting secondary structure in the mRNA is believed to lower the translation initiation rate in the absence of heat stress. Removal of these downstream sequences leads to elevated levels of expression in unstressed cells. It is conceivable that a similar mechanism involving interaction between the translation initiation region and the 3′ region, encompassing bases +515 to +786, is responsible for the repression of *katF* translation during growth in minimal medium.

The posttranscriptional regulation of *katF* differs in complex medium as compared to minimal glucose medium. In LB, all three sets of fusions showed identical patterns of regulation. This indicates that the posttranscriptional repression system active during growth in M9 does not operate in LB. It also indicates that there is a separate posttranscriptional induction system, since expression of the protein fusions increased some sixfold as cells entered the late log phase of growth. This induction cannot be due to an increase in *katF* transcription, since the protein fusions reached near-maximal levels before the expression of the operon fusions began to increase. It is possible that this posttranscriptional induction involves an increase in translation initiation and/or an increase in *katF* mRNA stability. One or both of these mechanisms may be regulated by KatF itself, since expression of the *katF* protein fusion is altered in a *katF*::Tn*10* background. These mechanisms have been implicated in the regulation of σ^{32} (3, 7, 23, 29).

In light of the central role that KatF plays both during nutrient starvation and in stress resistance in growing cells (8, 15, 16, 22), it is not surprising that it is regulated at many levels.

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