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# Regulation of Transcription of *katE* and *katF* in *Escherichia coli*

MICHAEL R. MULVEY, JACEK SWITALA, ANDREW BORYS, AND PETER C. LOEWEN*

*Department of Microbiology, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada*

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**Fusion plasmids with *lacZ* under the control of the *katE* (encoding catalase or hydroperoxidase HPII) and *katF* (encoding a sigma factor-like protein required for *katE* expression) promoters were constructed. Expression from both *katE* and *katF* promoters was low in rich medium but elevated in poor medium during log-phase growth. Furthermore, the slowdown in growth as cells entered the stationary phase in rich medium, a result of carbon source depletion, was associated with an increase in *katE* and *katF* expression. A simple reduction in the carbon source level as the cells entered the stationary phase was not responsible for the increase in expression, because transferring the culture to a medium with no glucose did not induce expression from either promoter. Spent rich medium from stationary-phase cells was capable of inducing expression, as were simple aromatic acids such as benzoate, *o*-hydroxybenzoate, and *p*-aminobenzoate added to new medium. Anaerobiosis did not cause an increase in expression, nor did it significantly change the pattern of expression. Regardless of the medium, *katF* expression was always turned on before or coincidently with *katE* expression; in the presence of benzoate *katF* was fully induced, whereas *katE* was only partially induced, suggesting that a factor in addition to KatF protein was involved in *katE* expression. During prolonged aerobic incubation, cells lacking *katF* died off more rapidly than did cells lacking either *katE* or *katG*.**

*Escherichia coli* produces two catalases or hydroperoxidases, the bifunctional catalase-peroxidase HPI and the monofunctional catalase HPII, encoded by the genes *katG* (10) and *katE* (6), respectively. Purification and physical characterization have shown that the two enzymes differ significantly from each other and from a typical catalase. HPI is active as a tetramer of 81,000-Da subunits and two protoheme IX groups (3), whereas HPII is active as a hexamer of 93,000-Da subunits and six heme *d*-like groups (1, 7).

The levels of the two catalases respond to different stimuli; HPI synthesis is induced by $H_2O_2$ added to the medium, and HPII synthesis is induced during growth into the stationary phase or during growth on tricarboxylic acid cycle intermediates (8). The mechanisms controlling these induction patterns have recently become the object of study in a number of laboratories. The *katG* gene has been shown to be part of the OxyR regulon, which responds to oxidative stress, including the presence of hydrogen peroxide (2, 25) as part of a broader response illicited by redox-cycling reagents (4). The mechanism controlling the expression of *katE* is quite different and requires a functional *katF* gene as a positive effector (9, 15, 20). Sequence analysis of *katF* has shown that KatF protein closely resembles known σ factors, suggesting that it may control the expression of a number of genes, including *katE* (14). The gene for exonuclease III, *xthA*, has been shown to be under the control of *katF* (18), and lesions in *katF* have been shown to result in sensitivity to broad-spectrum UV radiation (19), confirming this suggestion. Various metabolic changes have been studied as to their influence on *katE* expression, confirming that *katE* expression is turned on as cells enter the stationary phase, is unaffected by hydrogen peroxide, is unaffected by anaerobiosis, could be induced by a component of stationary-phase culture supernatant, and might be affected by intracellular pH (20). It has also been shown that a shift to anaerobic conditions induces HPII synthesis and that high concentrations of glucose repress HPII synthesis by a mechanism that is independent of cyclic AMP (12). From these results it is clear that the synthesis of HPII and, consequently, the mechanism involving *katF* are influenced by an as yet undefined factor that is dependent on the stage of cell growth. In this report we describe the construction of *katE*::*lacZ* and *katF*::*lacZ* fusions and their use in a study of the relationship of *katE* and *katF* expression to cell metabolism.

## MATERIALS AND METHODS

**Bacterial strains and plasmids.** Strain NM522 [*recA supE* Δ(*lac-proAB*) *hsd-5*(F′ *proAB lacI*$^q$ *lacZ*Δ*15*)] (11) was transformed with the various fusion plasmids and used as the host for β-galactosidase expression assays. Strain UM315 (NM522 but *katF13*::Tn*10*) was constructed by transduction of NM522 with a P1 lysate of UM122 (*katF13*::Tn*10*) (8) and selection for tetracycline-resistant colonies, which evolved oxygen much more slowly when tested with a drop of 30% $H_2O_2$. Strains MP180 (HfrH *thi-1*), UM120 (like MP180 but *katE12*::Tn*10*) (8), UM122 (like MP180 but *katF13*::Tn*10*) (8), and UM202 (like MP180 but *katG17*::Tn*10*) (8) were used as isogenic strains for survival studies. The fusion plasmid containing the *katE* promoter, pRSkatE16, was constructed by inserting the 1.4-kb *Sma*I-*Eco*RV fragment (Fig. 1a) of pAMkatE72, which has *katE* on a 3.0-kb *Pst*I-*Cla*I fragment (15) in the Bluescript KS+ vector (Stratagene Cloning Systems), into the *Sma*I site upstream of *lacZ* gene of pRS415 (21, 22). The fusion plasmid containing the *katF* promoter, pRSkatF5, was constructed by inserting the 600-bp *Eco*RI-*Dra*I fragment (Fig. 1b) from pMF2, which has *katF* on a 2.3-kb *Kpn*I fragment (14) in the Bluescript KS+ vector, into pRS415 cut with *Eco*RI and *Sma*I upstream of the *lacZ* gene.

**Growth media.** LB medium (13) containing 10 g of tryptone (GIBCO), 5 g of yeast extract (GIBCO), and 10 g of NaCl, in some cases supplemented with components specified below, was used as a rich medium. M9 minimal medium (13) containing 42 mM $Na_2HPO_4$, 22 mM $KH_2PO_4$, 9 mM NaCl, and 19 mM $NH_4Cl$ was supplemented after autoclaving with 1 mM $MgSO_4$, 1 μg of thiamine per ml, 1 ml of a

* Corresponding author.

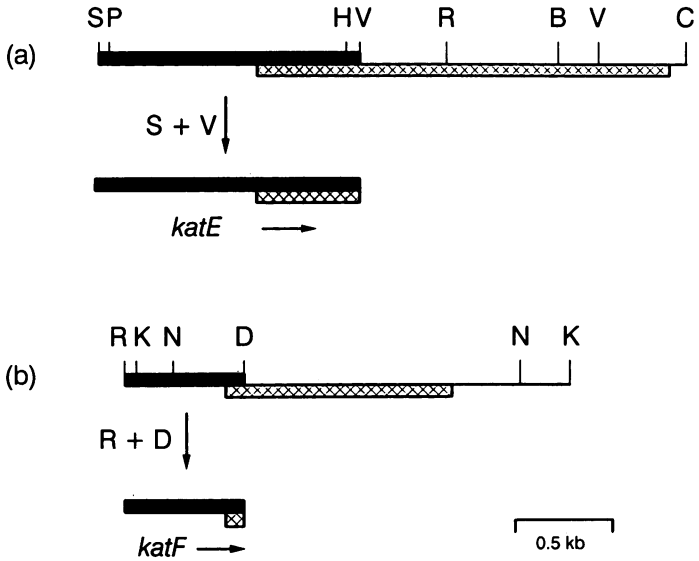


FIG. 1. Construction of fusion plasmids containing *lacZ* under the control of the *katE* (a) and *katF* (b) promoters. (a) A 1.4-kb fragment (solid broad line) was cut from pAMkatE72 with *Sma*I (S) and *Eco*RV (V) and ligated into the *Sma*I site of pRS415 to generate pRSkatE16. The direction of transcription from the *katE* promoter is indicated by the arrow. The cross-hatched region indicates the coding region of *katE*. (b) A 600-bp fragment (solid broad line) was cut from pMF2 with *Eco*RI (R) and *Dra*I (D) and ligated into pRS415 cut with *Eco*RI and *Sma*I to generate pRSkatF5. The direction of transcription from the *katF* promoter is indicated by the arrow. The cross-hatched region indicates the coding region of *katF*.

trace elements solution (2.5 g of $FeSO_4 \cdot 7H_2O$, 2.9 g of $H_3BO_3$, 1.2 g of $CoSO_4 \cdot 7H_2O$, 0.1 g of $CuSO_4 \cdot 5H_2O$, 0.09 g of $MnCl_2 \cdot 4H_2O$, 5.0 ml of $H_2SO_4$, 2.5 g of $Na_2MoO_4 \cdot 2H_2O$, 2.1 g of $ZnSO_4 \cdot 7H_2O$ in 1 liter of $H_2O$) per liter, and carbon sources as specified in individual experiments. The buffers MES [2-(*N*-morpholino)ethanesulfonic acid], PIPES [piperazine-*N*,*N*'-bis(2-ethanesulfonic acid)], and HEPES (*N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid) were obtained from Sigma. SM buffer contained 20 mM Tris hydrochloride (pH 7.6), 100 mM NaCl, 1 mM $MgSO_4$, and 0.1 g of gelatin per liter.

**Enzyme assays.** β-Galactosidase was assayed as described by Miller (13), except that treatment with 20 μl of chloroform and incubation for 4 min at 28°C were employed to lyse the cells. More chloroform or longer incubation times resulted in variations in the high activity levels observed in some experiments. β-Galactosidase activity is reported in Miller units. For changes in *lacZ* expression to be comparable despite significant differences in growth rates in the different media, the β-galactosidase data in Fig. 2 through 6 are presented as a function of cell density measured as $A_{600}$. The growth data are also presented in a format different from the norm. The $A_{600}$ (cell density) is plotted on the *x* axis, and time is on the *y* axis. This results in slow or no growth appearing as a vertical or rising line and rapid growth appearing as a horizontal line.

## RESULTS

**Isolation of fusion plasmids.** Fragments containing either the *katE* or *katF* promoters (Fig. 1) were inserted into the operon fusion vector pRS415. The nucleotide sequences of both genes confirmed that a portion of the coding regions and the upstream sequence sufficient for gene expression were contained on the cloned fragments (unpublished data for *katE*; reference 13 for *katF*). Because the cloned fragment in pRSkatE16 was quite large (1.3 kb), giving rise to the possibility of other promoters being present that might influence expression, the plasmids were transformed into strains NM522 and UM315, which are isogenic except for the transposon insertion in *katF* in UM315, and the levels of β-galactosidase were determined (Table 1). The low expression from the *katE* fusion in UM315 as compared with that in NM522 confirmed that expression from the cloned promoter remained under the positive control of *katF*, which was missing in UM315. It was also evident from these data that initiation from the *katE* promoter was six to seven times better than initiation from the *katF* promoter when fully induced. Because the amount of plasmid DNA was essentially the same in pRSkatE16 and pRSkatF5 transformants, the differences in expression were not the result of differences in plasmid copy number but probably reflected different promoter efficiencies. Catalase expression in NM522 and UM315 was not affected by the presence of multicopy plasmids containing *katE* and *katF* promoters, confirming that gene expression remained normal in the transformed strains; this is consistent with the normal expression patterns of HPII from plasmids carrying the whole *katE* gene (15).

TABLE 1. Effect of *katF* deficiency on the expression of *lacZ* from fusion plasmids pRSkatE16 and pRSkatF5 in either NM522 or UM315 grown for 18 h in LB medium to an $A_{600}$ of >4.5

| Strain and plasmid | β-Galactosidase (Miller units) |
|---|---|
| NM522 (wild type) | ND[a] |
| NM522(pRSkatE16) | 14,568 |
| NM522(pRSkatF5) | 3,993 |
| UM315 (*katF*::Tn*10*) | ND |
| UM315(pRSkatE16) | 194 |
| UM315(pRSkatF5) | 4,196 |

[a] ND, Not detectable.

**Effect of carbon source on *katE* and *katF* expression.** The carbon source has been shown to affect the level of HPII in *E. coli* (8, 12), and we investigated the effect of growth medium on the expression of *katE* and *katF*. In LB medium the level of expression from the *katE* promoter remained low during exponential-phase growth but increased significantly as the cells approached the stationary phase (Fig. 2). The changes in β-galactosidase levels controlled by the *katE* promoter essentially paralleled the changes in HPII levels observed during a similar growth experiment (8). Expression from the *katF* promoter differed in that it commenced in the early-exponential phase and increased across the growth curve into the stationary phase to a maximum level that was approximately one-third of the level of *katE* expression.

In minimal medium with either glucose or succinate as the carbon source, there was a rapid increase in expression from both promoters very early in the exponential phase to a steady-state level that was maintained throughout growth into the stationary phase (Fig. 3). The low initial levels of β-galactosidase in these cultures were the result of using an inoculum from a mid-log-phase culture grown in LB medium. If an inoculum with high β-galactosidase levels from stationary-phase cells in either LB medium or glucose-minimal medium was used, the enzyme levels in both glucose- and succinate-minimal media remained at a high level throughout growth. There was a significant difference

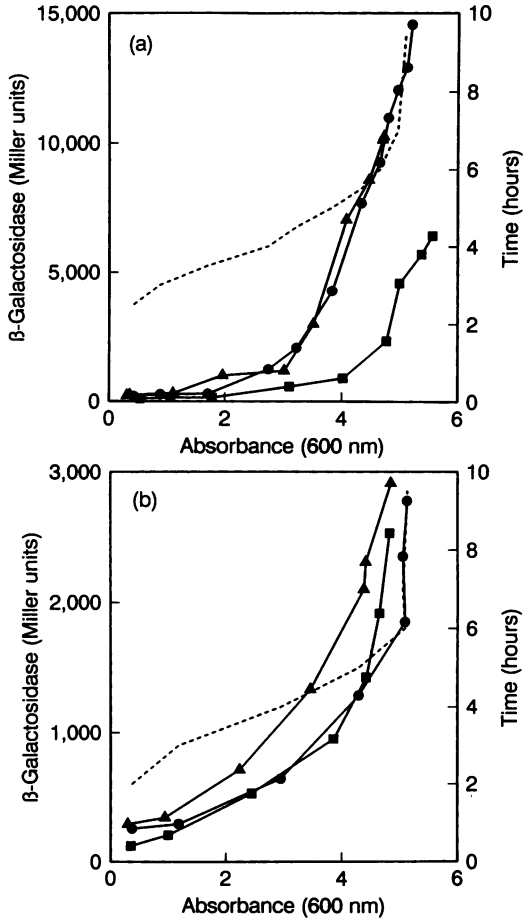


FIG. 2. β-Galactosidase synthesis from *lacZ* under the control of the *katE* promoter (pRSkatE16) (a) and the *katF* promoter (pRSkatF5) (b) expressed as a function of cell density ($A_{600}$). β-Galactosidase was assayed in cultures growing in LB medium buffered at pH 6.0 (■) with 100 mM MES, at pH 7.0 (●) with 100 mM PIPES, and at pH 8.0 (▲) with 100 mM HEPES. Growth at pH 7 is indicated by the dashed line, which represents cell density ($A_{600}$) as a function of time. Note the unusual orientation of the growth curve graph, which results in slow growth being represented by a nearly vertical line and rapid growth being represented by a nearly horizontal line. Growth curves at the other pHs were very similar and are not shown.

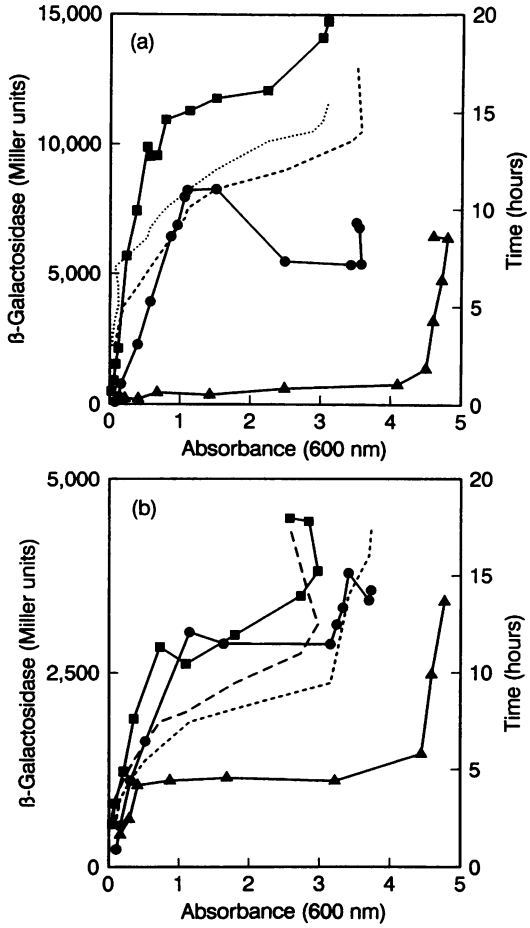


FIG. 3. β-Galactosidase synthesis from *lacZ* under the control of the *katE* promoter (pRSkatE16) (a) and the *katF* promoter (pRSkatF5) (b) expressed as a function of cell density ($A_{600}$). β-Galactosidase was assayed in cultures growing in M9 minimal medium supplemented with 20 mM glucose (●), 20 mM succinate (■), or 20 mM glucose and 0.2% (wt/vol) of a mixture of 20 amino acids (▲). Growth curves for the glucose (– – –) and succinate (-----) cultures represent cell density ($A_{600}$) as a function of time.

in *katE* but not *katF* expression between glucose- and succinate-containing media; succinate induced a level two-fold higher than that induced by glucose. When combined with the observation that *katF* expression is turned on before *katE* expression during growth, the differential effect of the carbon source suggested that a factor in addition to KatF protein was involved in the expression of *katE*.

Amino acids are a major component of LB medium, and the addition of a mixture of all 20 amino acids to glucose-minimal medium repressed expression from both promoters until growth approached the stationary phase (Fig. 3). When amino acids were used as the sole carbon source, expression was slightly elevated throughout growth (Table 2) but increased to a high level during the stationary phase, indicating that a combination of both glucose and amino acids was required to maintain low levels of expression. The addition of a single amino acid, either aspartate (acidic), alanine (neutral), or arginine (basic), at the concentration of 0.2% (wt/vol) to glucose-minimal medium did not serve to lower expression, suggesting that it was not a simple buffering effect that was repressing expression when the amino acids were present (Table 2).

Glucose has been reported to lower the levels of HPII

TABLE 2. Effect of amino acids in minimal medium on *katE* and *katF* expression

| Medium[a] | β-Galactosidase (Miller units) | |
|---|---|---|
| | pRSkatE16 | pRSkatF5 |
| Glucose | 4,005 (0.87)[b] | 3,040 (1.03) |
| Amino acid mixture | 1,469 (0.58) | 1,116 (0.49) |
| Glucose + amino acid mixture | 848 (0.61) | 839 (0.39) |
| Glucose + aspartate | 5,278 (0.88) | 2,944 (0.82) |
| Glucose + alanine | 5,406 (0.69) | 2,722 (0.66) |
| Glucose + arginine | 6,245 (1.20) | 2,760 (0.72) |

[a] The following concentrations of components were used: glucose, 16 mM; amino acid mixture, 0.2% (wt/vol) of a mixture containing equal amounts of 20 common amino acids; aspartate, alanine, and arginine, 0.2% (wt/vol).

[b] The values within parentheses are the $A_{600}$ readings of cell density. Cells growing in M9-glucose medium grew to an $A_{600}$ of 3.4 in the stationary phase. All of the cultures in this table were in the midlog phase.

TABLE 3. Effect of glucose, glycerol, and lactose on *katE* and *katF* expression in various media

| Medium | β-Galactosidase (Miller units) | |
|---|---|---|
| | pRSkatE16 | pRSkatF5 |
| M9 + glucose (0.8 mM) | 9,783 (0.36)[a] | 2,864 (0.35) |
| M9 + glucose (16 mM) | 8,283 (1.05) | 3,049 (0.99) |
| M9 + glucose (16 mM) | 6,068 (3.41) | 3,302 (3.43) |
| M9 + glucose (100 mM) | 5,631 (0.57) | NT[b] |
| LB | 7,323 (3.86) | NT |
| LB + glucose (100 mM) | 985 (4.90) | NT |
| LB + glycerol (200 mM) | 2,618 (5.57) | NT |
| LB + lactose (50 mM) | 973 (6.46) | NT |
| LB (pH 7)[c] | 8,339 (4.74) | 2,695 (4.74) |
| LB (pH 7) + glucose (100 mM) | 7,010 (9.39) | 3,079 (7.14) |
| LB (pH 7) + glycerol (200 mM) | 6,423 (8.77) | 3,734 (9.28) |
| LB (pH 7) + lactose (50 mM) | 5,729 (6.98) | 3,597 (8.28) |

[a] The values within parentheses are the $A_{600}$ readings of cell density. Cells growing on 16 mM glucose grew to an $A_{600}$ of 3.4 in the stationary phase, whereas cells growing on 0.8 mM glucose reached the stationary phase at an $A_{600}$ of 0.36.
[b] NT, Not tested.
[c] The medium was adjusted to pH 7 with 100 mM PIPES.

found in *E. coli* (12); as noted above, there was a twofold difference in expression of *katE* in cultures grown on glucose and succinate in minimal medium. When a lower concentration of glucose (0.8 mM) was used, such that the cells entered the stationary phase at a lower cell density, the expression of both *katE* and *katF* promoters increased to the same extent as that in cultures with 16 mM glucose (Table 3). In unbuffered LB medium the effect of 100 mM glucose was to prevent any increase in *katE* expression (Table 3), consistent with the glucose effect observed by other workers (12). However, 50 mM lactose also prevented the increase in expression, and 200 mM glycerol was effective in reducing expression, suggesting that a more general phenomenon related to growth on high concentrations of any carbon source was involved. Cultures growing on the high-concentration carbon source in unbuffered medium grew more slowly and did not grow beyond a cell density equivalent to that achieved in unsupplemented media, possibly because of a drop in pH of the medium. When the LB medium was buffered at pH 7.0, supplements of 16, 50, or 100 mM glucose did not affect the final level of expression from *katF* and only slightly reduced the level of expression from *katE*. The cells grew to a much higher cell density, and the turn-on of expression was delayed to approximately the same time before entry into the stationary phase (Fig. 4). Consequently, the low expression of *katE* and *katF* in unbuffered media can be attributed to pH inhibition of normal growth patterns, including gene expression associated with cells entering the stationary phase.

**Effect of starvation on *katE* and *katF* expression.** One possible explanation for the increases in *katE* and *katF* expression before the stationary phase or when both glucose and amino acids are not jointly present is that cell starvation for a specific metabolite acts as a signal to turn on expression. To test this idea, starvation was induced by transferring cells from a midlog culture grown on glucose and amino acids to a new medium lacking either carbon (ammonium ion present as the nitrogen source) or ammonia (glucose present as the carbon source). Cell growth was prevented in both cases, but only carbon starvation prevented *katE* expression and lowered *katF* expression. Starvation for ammonia did not reduce expression from either promoter despite the lack

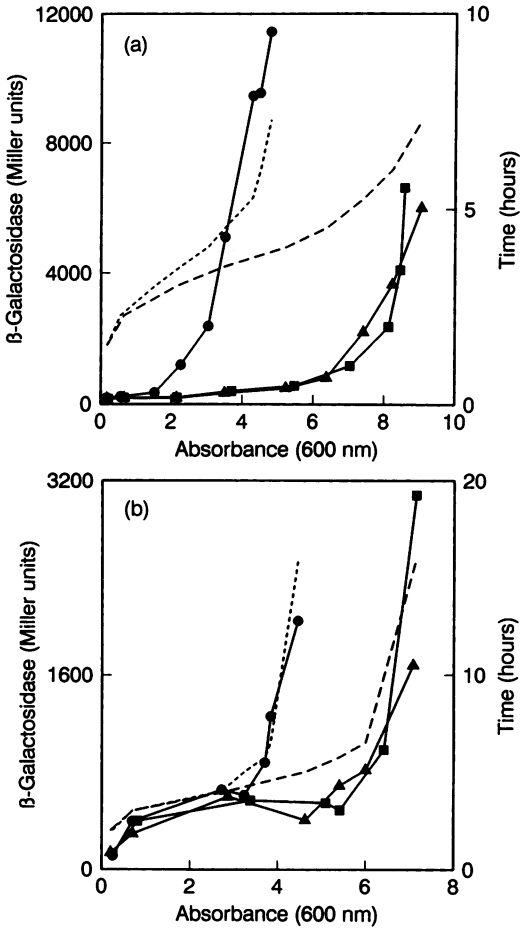


FIG. 4. Effect of high glucose on β-galactosidase synthesis from *lacZ* under the control of the *katE* promoter (pRSkatE16) (a) and the *katF* promoter (pRSkatF5) (b) expressed as a function of cell density ($A_{600}$). β-Galactosidase was assayed in cultures growing in LB medium (●) or LB medium supplemented with 16 mM glucose (■) or 100 mM glucose (▲). All media were adjusted to pH 7.0 with 100 mM PIPES buffer before inoculation. Growth curves in LB medium (----) and LB supplemented with 100 mM glucose (– – –) represent cell density ($A_{600}$) as a function of time. The growth curve in medium supplemented with 16 mM glucose was the same as that shown for 100 mM glucose.

of growth (Table 4). Because the removal of all sources of carbon from the medium did not induce expression, whereas starvation arising from depletion of a medium component during growth into the stationary phase did induce expres-

TABLE 4. Effect of removing all carbon or nitrogen from M9 medium on *katE* and *katF* expression

| Medium[a] | β-Galactosidase (Miller units) | |
|---|---|---|
| | pRSkatE16 | pRSkatF5 |
| M9 + glucose + $NH_4Cl$ | 6,951 (3.8)[b] | 2,853 (3.7) |
| M9 + $NH_4Cl$ | 311 (0.08) | 1,264 (0.13) |
| M9 + glucose | 5,613 (0.10) | 2,781 (0.15) |

[a] The following concentrations of supplements were used: glucose, 16 mM; $NH_4Cl$, 19 mM; of Casamino Acids, 0.006% (wt/vol) (present in each experiment).
[b] The numbers within parentheses are the $A_{600}$ values of cell density after incubation for 20 h.

TABLE 5. Effect of spent LB medium on *katE* and *katF* expression

| Medium | β-Galactosidase (Miller units) | |
|---|---|---|
| | pRSkatE16 | pRSkatF5 |
| LB | 102 (0.42)[a] | 229 (0.45) |
| LB (spent) | 1,616 (0.57) | 1,074 (0.54) |
| LB (spent, dialyzed for 16 h) | 162 (0.55) | 628 (0.58) |
| LB (spent, boiled for 3 min) | 2,138 (0.43) | NT[b] |

[a] The numbers within parentheses are the cell densities in $A_{600}$ units at which the assay was carried out. In LB medium, the cultures were assayed after 2 h, whereas in spent media, the cultures were incubated for 5 h. Cells growing in LB medium reached the stationary phase at an $A_{600}$ of approximately 5.0.

[b] NT, Not tested.

sion, it can be surmised that some form of carbon metabolism, even in the absence of cell growth, is required for induction, possibly through the production of an inducing component.

This conclusion is consistent with the observation (20) that a component of spent medium was responsible for the induction of *katE* expression. We have repeated this latter experiment, confirming that spent medium from a stationary-phase culture caused an induction of *katE* expression and showing that it also induces *katF* expression (Table 5). When the spent medium was dialyzed against SM buffer before the addition of new cells, the extent of induction was significantly reduced, indicating that the active component had been inactivated by dialysis or was relatively small and had passed out of the dialysis bag. Storage of the spent medium for 48 h at 5°C or boiling did not reduce its inducing ability. Attempts to identify the component by adding to LB medium various metabolic components that had previously been associated with elevated levels of HPII (8), including lactate, acetate, pyruvate, malate, succinate, and fumarate, were unsuccessful; none of the components had any effect on levels of expression (Table 6). The nonmetabolizable weak acids benzoate and *o*-hydroxybenzoate did induce an increase in expression, possibly related to induced changes in internal pH (23). The closely related metabolizable acid *p*-aminobenzoate also caused induction, but its isomer, *o*-aminobenzoate, did not. Whether there is any relationship between these aromatic acids and the active components in spent medium is under investigation.

TABLE 6. Effect of various supplements on *katE* expression in pRSkatE16

| Medium[a] | β-Galactosidase (Miller units) |
|---|---|
| LB | 244 (0.76)[b] |
| LB + acetate | 179 (0.54) |
| LB + KF | 213 (0.45) |
| LB + malate | 275 (0.57) |
| LB + succinate | 252 (0.57) |
| LB + fumarate | 251 (0.74) |
| LB + glutamate | 240 (0.76) |
| LB + phenylalanine | 469 (0.84) |
| LB + benzoate | 3,155 (0.80) |
| LB + *o*-hydroxybenzoate | 6,684 (0.10) |
| LB + *p*-aminobenzoate | 6,851 (0.66) |
| LB + *o*-aminobenzoate | 544 (0.66) |

[a] All supplements were added to 20 mM except *o*-hydroxybenzoate, which was at 10 mM, and KF, which was at 5 mM. The medium was buffered to pH 7 in the presence of 100 mM PIPES.

[b] The numbers within parentheses are the cell densities in $A_{600}$ units at the time of assaying for β-galactosidase. Stationary phase in LB medium was reached at an $A_{600}$ of approximately 5.0.

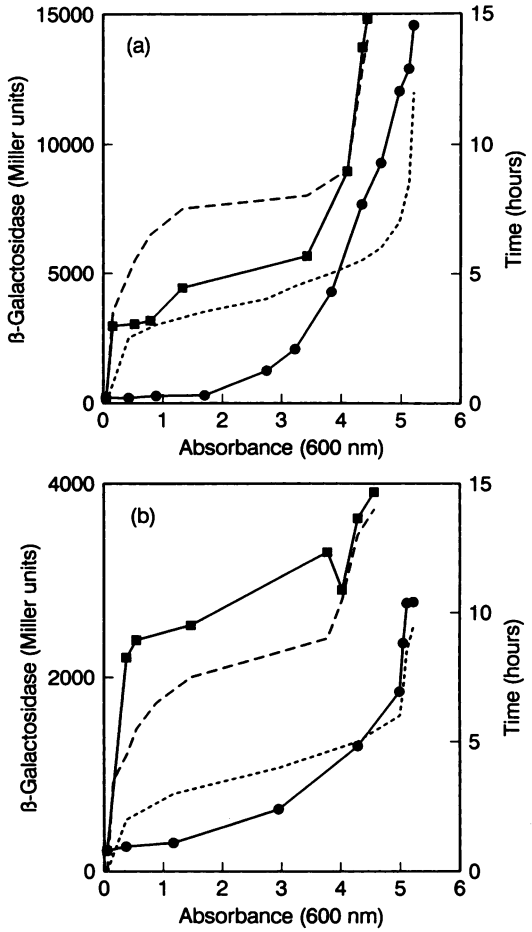


FIG. 5. Effect of benzoate on β-galactosidase synthesis from *lacZ* under the control of the *katE* promoter (pRSkatE16) (a) and the *katF* promoter (pRSkatF5) (b) expressed as a function of cell density ($A_{600}$). β-Galactosidase was assayed in cultures grown in LB medium (●) and LB medium with sodium benzoate added to final concentration of 20 mM before inoculation (■). Growth curves without (----) and with (– – –) benzoate represent cell density ($A_{600}$) as a function of time.

**Effect of pH changes on *katE* and *katF* expression.** Schellhorn and Hassan (20) have proposed that there may be a link between the induction of an acid-sensitive regulon by nonmetabolizable weak acids such as benzoate and the pH independence of HPII expression. As described above, we have confirmed that benzoate (Fig. 5) and *o*-hydroxybenzoate (Table 6) are potent inducers of expression of both *katE* and *katF* promoters. In contrast, KF and the common tricarboxylic acid cycle acids, including acetate, which are known to lower internal pH (17), did not have any effect on expression. At least two explanations are possible for this contradiction: (i) pH changes are responsible for the induction, and the aromatic amino acids are more effective at changing the internal pH than acetate or KF; and (ii) pH changes are not responsible, and the inducing effect of the aromatic acids is a result of their structural similarity to a putative inducer molecule. The fact that one metabolizable aromatic acid, *p*-aminobenzoate, but not a second, *o*-aminobenzoate, is an effective inducer of *katF* expression suggests one possible avenue to pursue. The effect of external

pH on expression was also studied and revealed little change in the expression of *katF* at pH 7 and 8, but a significant delay in turn-on and reduction in *katE* expression was observed at pH 6 (Fig. 2).

**Effect of anaerobiosis on *katE* and *katF* expression.** The literature contains conflicting reports of the effect of anaerobiosis on the expression of HPII. Little HPII synthesis was observed during anaerobic growth in minimal medium with nitrate or fumarate as the terminal electron source (8), whereas it has been observed that the synthesis of HPII during anaerobic growth in LB medium increased as the cells entered the stationary phase in a pattern similar to that observed for aerobic cultures but to a level that was only one-third of the induced aerobic level (20). It has also been reported that HPII synthesis was induced within an hour of shifting a culture to anaerobic conditions in LB medium; from this, they concluded that the increase in HPII levels in aerobic stationary-phase cells was the result of anaerobiosis during the onset of the stationary phase (12). Because an anaerobic effect was not consistent with the model involving pH or metabolite signals suggested above, we investigated the effect of anaerobiosis on *katE* and *katF* expression. Shifting cultures to anaerobic growth did not cause an increase in expression from either promoter even after 3 h (Fig. 6), but there was a turn-on of expression as the cells approached the stationary phase. Furthermore, we assayed the level of oxygen in an aerobically growing culture and found that the oxygen concentration was 165 μM throughout growth; when the cells had entered the stationary phase, the oxygen concentration was 195 μM, which is only slightly lower than 225 μM, the maximum oxygen concentration in water saturated with air. This would argue against the turn-on of HPII synthesis being a result of anaerobiosis, and we have concluded that anaerobiosis does not have a direct effect on the expression of these two promoters.

**Effect of *katE*, *katF*, and *katG* on cell survival.** It is clear that *katE* and *katF* are expressed in the stationary phase or during growth on less-than-ideal media, in part to protect the cell against hydrogen peroxide and other harmful metabolites. We investigated the relative importance of the three genes affecting catalase levels, *katE*, *katF*, and *katG*, on the survival of cultures shaken vigorously in glucose minimal medium for a period of up to 10 days; the results from the first 5 days are shown in Fig. 7. The strains used were all derived from the same parent strain, MP180, and differed only in having individual transposon insertions in *katE* (UM120), *katF* (UM122), or *katG* (UM202). Survival was poorest when *katF* was defective, and survival increased in the order *katE*::Tn*10* < *katG*::Tn*10* < wild type. Clearly, *katF* is affecting the synthesis of gene products in addition to HPII (from *katE*) that are important for the survival of dormant or stationary-phase cells.

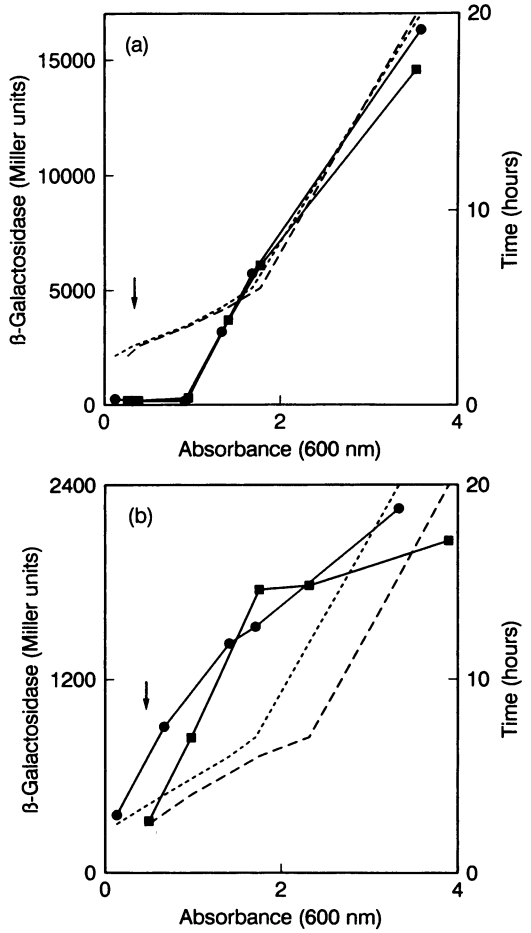


FIG. 6. Effect of anaerobic growth on β-galactosidase synthesis from *lacZ* under the control of *katE* (pRSkatE16) (a) and *katF* (pRSkatF5) (b) expressed as a function of cell density ($A_{600}$). β-Galactosidase was assayed in cultures grown in LB medium flushed with nitrogen and sealed (●) and in cultures grown aerobically for 2 h to an $A_{600}$ of approximately 0.2 (at the point indicated by the arrow) and then flushed thoroughly with nitrogen and sealed (■). Growth in the anaerobic cultures (– – –) and in the cultures shifted to anaerobic growth (-----) represent cell density ($A_{600}$) as a function of time.

## DISCUSSION

Fusion plasmids in which *lacZ* transcription is controlled from either *katE* or *katF* promoters have been used to study the effects of various growth conditions, including rich and minimal media, high glucose concentrations, anaerobiosis, and various medium supplements, on *katE* and *katF* expression. Transcription from both promoters was turned on during growth on poor media and during the beginning of the stationary phase in rich media, suggesting a link with the starvation response. Strategies for survival of starvation conditions have been the subject of study for several years, and a series of genes that are turned on during the starvation process has been identified in *E. coli* (26) and *Salmonella typhimurium* (24) and shown to be linked to the osmotic shock response (5). Not only are the patterns of expression of *katE* and *katF*, with induction occurring when the cell experiences suboptimal growth conditions, similar to the pattern of gene expression resulting from the starvation response, but their gene products seem to play an important role in the ability of a cell to survive starvation inactivation. In particular, inactivation of KatF protein significantly reduced the ability of cells to survive prolonged exposure to starvation conditions in a fashion remarkably similar to the effect of mutations inactivating several peptidases that are also required for survival (16). That KatF is more important than either HPII or exonuclease III (unpublished data) in protecting the cell during starvation is consistent with its role as a σ factor responsible for turning on the transcription of other genes, the products of which are also required for long-term cell viability. Determination of the number and identity of these other genes controlled by *katF* will help to

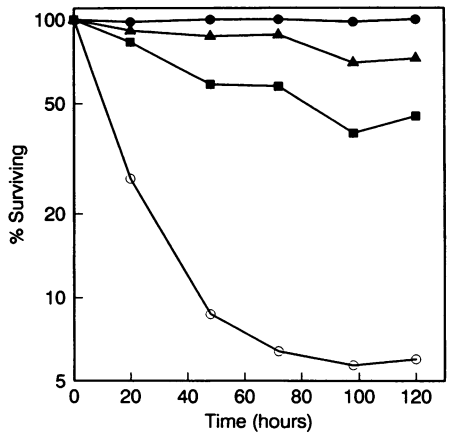


FIG. 7. Effect of lesions in the various catalase genes on survival during long incubation at 37°C under aerobic conditions. Strains MP180 (wild type) (●), UM202 (*katG*::Tn*10*) (▲), UM120 (*katE*::Tn*10*) (■), and UM122 (*katF*::Tn*10*) (○) were grown for 20 h in minimal medium supplemented with 5 mM glucose with shaking at 37°C. At this time (0 h on the graph) and at subsequent times, samples were removed for determination of the viable cell count plating on LB-agar plates.

clarify the importance of KatF in the starvation and osmotic shock responses.

Expression from the *katF* promoter is turned on before or coordinately with expression from the *katE* promoter, consistent with KatF protein being required for maximal expression of *katE*. It has been demonstrated that *katF* is required for the expression of *katE* (15) and *xthA* (18), and evidence in this report suggests that other genes involved in protecting the cell against starvation are also controlled by *katF*. The nucleotide sequence of *katF* has revealed a protein sequence with strong homology to known σ factors (14), suggesting that the positive regulatory role of KatF protein (15, 20) lies in its ability to direct RNA polymerase to a series of genes encoding proteins required for protection of the cell against starvation and near-UV radiation. Previously it had been shown that aromatic amino acids turned on expression of *katE*, leading to the suggestion that *katE* expression was responding to changes in internal pH (20). We have shown that it is, in fact, *katF* expression that is turned on by the aromatic amino acids and that KatF protein, in its role as a σ factor, activates *katE* transcription. However, it remains to be determined whether it is the internal pH change caused by the aromatic amino acids or the structural similarity of the acids to an intracellular inducer molecule that normally accumulates during periods of slow growth that is responsible for the activation of *katF* expression.

Whereas there was only one induced level of *katF* expression, there were at least two distinct levels of *katE* expression. Maximal induction of *katF*, such as occurs in the presence of benzoic acid in LB medium or glucose in minimal medium, resulted in a 10-fold increase in the level of *katE* expression. The subsequent three- or fourfold increase in *katE* expression to its maximal level, such as occurred during the shift to the stationary phase in LB-benzoate medium, occurred without any change in *katF* expression, suggesting that at least one further factor was involved in maximizing *katE* expression. It remains to be determined whether the additional factor acts directly to facilitate RNA polymerase binding, thereby stimulating *katE* expression, or indirectly by, for example, modifying KatF protein to make it a more efficient σ factor, thereby increasing the affinity of the RNA polymerase for the *katE* promoter.

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