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Transcriptional Regulation of *katE* in *Escherichia coli* K-12†

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***Escherichia coli* produces two distinct species of catalase, hydroperoxidases I and II, which differ in kinetic properties and regulation. To further examine catalase regulation, a *lacZ* fusion was placed into one of the genes that is involved in catalase synthesis. Transductional mapping revealed the fusion to be either allelic with or very close to *katE*, a locus which together with *katF* controls the synthesis of the aerobically inducible hydroperoxidase (hydroperoxidase II). *katE* was expressed under anaerobic conditions at levels that were approximately one-fourth of those found in aerobically grown cells and was found to be induced to higher levels in early-stationary-phase cells relative to levels of exponentially growing cells under both anaerobic and aerobic conditions. *katE* was fully expressed in air and was not further induced when the growth medium was sparged with 100% oxygen. Expression of *katE* was unaffected by the addition of hydrogen peroxide or by the presence of additional lesions in *oxyR* or *sodA*, indicating that it is not part of the *oxyR* regulon. When *katF*::Tn*10* was introduced into a *katE*::*lacZ* strain, β-galactosidase synthesis was largely eliminated and was no longer inducible, suggesting that *katF* is a positive regulator of *katE* expression.**

Escherichia coli produces two species of hydroperoxidase (HPI and HPII) (EC 1.11.1.6) whose syntheses are genetically controlled by three noncontiguous loci (*katE*, *katF*, and *katG*) (14, 16, 17). HPI, which is encoded by *katG*, is hydrogen peroxide inducible (3, 17, 22) and is produced in the absence of oxygen (11). In addition, HPI has a low K_m for hydrogen peroxide (4, 23) and possesses dual catalase and *ortho*-dianisidine peroxidase activities (4). In contrast, HPII has a high K_m (5), is induced by aeration (11), is not known to be produced anaerobically, and has no peroxidase activity (5). HPII synthesis increases dramatically when aerobically grown cells enter the stationary phase (11, 15), but the metabolic signal responsible for this increase has not been identified.

Increased levels of HPII have been correlated to both an increase in respiratory activity (11) and to the accumulation of tricarboxylic acid cycle intermediates (15). Two genes, *katE* and *katF*, controlling HPII synthesis have been identified (14, 16), but the relationship between them remains unclear. It is possible that one locus has a regulatory function while the other is a structural gene or that the two genes code for different subunits of the tetrameric enzyme. While a mutation in either locus eliminates the HPII function, only mutations in *katF* render the cell hypersensitive to near-UV light (8, 24). *katF* has recently been shown to be coallelic with *nur* (24), a gene which has previously been shown to confer protection from near-UV light (27).

Because of the two gene-one product nature of HPII biosynthesis, enzymatic assays cannot be used to monitor expression of either *katE* or *katF* directly, so alternative means of measuring gene expression must be employed. In this study we placed a *lacZ* operon fusion into the *katE* gene and transduced the fusion into various genetic backgrounds to determine whether its expression is subject to regulation by *katF*, OxyR, a hydrogen peroxide-inducible regulatory protein (3), or both *katF* and OxyR. We also examined expression as a function of growth phase and aeration. The elimination of a functional HPII also permitted us to examine the activity of the HPI protein independently of and in parallel with the activity of *katE*::*lacZ*.

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MATERIALS AND METHODS

Bacterial and phage strains. The bacterial and phage strains used in this study are listed in Table 1.

Chemicals. All chemicals were supplied by either Fisher Scientific Ltd. or Sigma Chemical Co. (St. Louis, Mo.). Stock solutions of chloramphenicol, kanamycin, streptomycin, and tetracycline were filter-sterilized (pore size, 0.4 μm; Acrodisc; Millipore Corp., Bedford, Mass.) and used as recommended previously (25).

Media. Minimal medium was the E medium described by Vogel and Bonner (28) and was supplemented with thiamine (final concentration, 20 μg/ml) and glucose (0.5%; wt/vol) (GMM). Glucose-yeast extract-amino acid (GYA) medium was identical to the glucose minimal medium, except that it was supplemented with 0.1% yeast extract and 20 amino acids at 10 μg/ml each. The rich medium used was Luria-Bertani (LB) broth (20), which contained the following per liter: 10 g of tryptone (Difco Laboratories, Detroit, Mich.), 5 g of yeast extract (Difco), and 10 g of NaCl. In some cases, LB broth was buffered with 100 mM PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)] and adjusted to pH 7.0. Solid media were solidified with 15 g of agar (Difco) per liter.

Isolation and mapping of a *katE*::*lacZ* mutant. Mutagenesis of *E. coli* SE5000 was conducted as described previously (2). The fusion and helper phage were added simultaneously to a mid-logarithmic-phase (optical density at 600 nm [OD_{600}], 0.6) culture of the host strain at a multiplicity of infection of 1:10. After a 30-min adsorption period at 37°C, the cells were washed 3 times to remove unadsorbed phage, and appropriate dilutions were plated onto LB medium containing 50 μg of kanamycin per ml to select for lysogens. The fusion phage used in this study possesses part of the *trp* operon in its genome, and thus occasionally integrates into this operon rather than the target gene during P1-mediated transduction (2). To preclude this possibility, all kanamycin-resistant transductants were selected on minimal medium that was devoid of tryptophan.

The absence of catalase activity in mutant colonies was scored as described previously (14) by applying a drop of

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

Strain or phage	Genotype	Source or reference
Bacteria		
SE5000	*araD139* Δ(*argF-lac*)U169 *rpsL150 relA1 deoC1 ptsF25 rbsR flb-5301 recA*	G. Weinstock (2)
NC1	Like SE5000, but *katE*::λp*lac*Mu53	This study
GC4468	Δ*lac4169 rpsL*	D. Touati
UM1	*lacY rpsL thi-1 katE1 kat-1* (*katG*?)	P. Loewen (16)
QC781	Like GC4468, but φ(*sodA*::MPR13)*25*	D. Touati
BW6160	Hfr *zdh-57*::Tn*10 metB1 relA1 spoT1*	B. L. Wanner (29)
RK4936	*araD139* Δ(*argF-lac*)*205 flbB5301* non-*gyrA219 relA1 rpsL150 metE70 btuB*::Tn*10*	B. N. Ames (3)
DF920	Hfr *tonA22 ompF627 pfkB*::Tn*10 relA1 pit-10 spoT1*	B. Bachmann
TA4112	Like RK4936, but *oxy*Δ3 [*oxy* (*oxyRbtuB*)*3*]	B. N. Ames (3)
NC4468	Like GC4468, but *katE*::*lacZ*	P1(NC1) × GC4468 → Kan^r
DTAK	Like QC781, but *katE*::*lacZ*	P1(NC1) × QC781 → Kan^r
NC4936	Like RK4936, but *katE*::*lacZ*	P1(NC1) × RK4936 → Kan^r
NC4112	Like TA4112, but *katE*::*lacZ*	P1(NC1) × TA4112 → Kan^r
UM120	*thi-1* HfrH *katE*::Tn*10*	P. Loewen (16)
GC120	Like GC4468, but *katE*::Tn*10*	P1(UM120) × GC 4468 → Tet^r
UM122	*thi-1* HfrH *katF*::Tn*10*	P. Loewen (16)
GC122	Like GC4468, but *katF*::Tn*10*	P1(UM122) × GC4468 → Tet^r
NC122	Like NC4468, but *katF*::Tn*10*	P1(UM122) × NC4468 → Tet^r
UM202	*thi-1* HfrH *katG*::Tn*10*	P. Loewen (15)
GC202	Like GC4468, but *katG*::Tn*10*	P1(UM202) × GC4468 → Tet^r
NC202	Like NC4468, but *katG*::Tn*10*	P1(UM202) × NC4468 → Tet^r
Phage		
λ p*lac*Mu53	*imm 'trp' 'lacZ*$^+$ *lacY*$^+$* *lacA' 'uvrD' xho*::*kan* Mu(cI ts*ner*$^+$*A*$^+$*'S*)	G. Weinstock (2)
λ pMu507	*c*I ts857 *Sam7* Mu *A*$^+$ *B*$^+$	G. Weinstock (2)
P1*vir*		S. Short

30% hydrogen peroxide against one side of each colony and scoring for the lack of oxygen evolution. Presumptive catalase-negative mutants were immediately picked from the other side of the colony (i.e., the side not treated with hydrogen peroxide) and streaked onto fresh plates. P1-mediated transductions were performed as described previously (25).

Growth conditions. Overnight cultures were grown in medium with the same composition as that used in the relevant experiment and were supplemented with antibiotics as appropriate. Aerobically incubated experimental cultures were grown in culture flasks (Bellco Glass, Inc., Vineland, N.J.) at 200 rpm (Lab-Line orbital shaker) at 37°C. To ensure good aeration, the culture/flask ratio was 1/5 in all cases. Growth was monitored by measuring the OD_{600} with a dual-beam spectrophotometer (model 810; Kontron).

Anaerobiosis was maintained by incubating cultures in a Coy anaerobic chamber as described previously (21) under an atmosphere of 5% CO_2–10% H_2–85% N_2. All media and cultures were preequilibrated in the chamber for at least 48 h prior to use.

Preparation of cell extracts. Culture samples were washed 3 times by centrifugation (10,000 × *g* for 10 min at 4°C) in cold 0.05 M potassium phosphate buffer (pH 7.0) containing 0.1 mM $MgSO_4$ and 150 μg of chloramphenicol per ml (to prevent further biosynthesis). Suspended cells were then sonicated (8 times for 45 s each time at 60 W) by using a cell disruptor (model W-370; Heat Systems Inc., Plainview, N.Y.) and centrifuged (27,000 × *g* for 30 min at 4°C) to remove cell debris.

In cases in which catalase and β-galactosidase were measured in whole cells, samples were washed as described above to eliminate interference by the medium in the assays. Chloramphenicol (final concentration, 150 μg/ml) was added to anaerobic samples following the growth period, to prevent de novo protein synthesis when the samples were removed from the anaerobic chamber.

Enzymatic assays. Catalase was measured in whole cells and crude cell extracts by measuring the decomposition of hydrogen peroxide at 240 nm (1). One unit is defined as 1 μmol of hydrogen peroxide hydrolyzed per min. β-Galactosidase assays were performed as described by Miller (20). One unit is defined as 1 nmol of *ortho*-nitrophenyl-β-galactoside hydrolyzed per min.

Protein concentration in cell extracts was measured by the method described by Lowry et al. (18) by using bovine serum albumin as the standard. In the case of whole-cell assays, the protein concentration was estimated from the optical density by assuming 0.107 mg of protein per ml per OD_{600} (20).

Nondenaturing polyacrylamide gel electrophoresis. Catalase isozymes were resolved and identified on nondenaturing 10% polyacrylamide gels as described previously (6), except the stacking gel was omitted.

RESULTS

Isolation and mapping of a *katE*::*lacZ* fusion mutant. Approximately 3,000 transductant colonies were tested for catalase activity. Three presumptive catalase mutants were identified and one (NC1) was also found to express β-galactosidase. When cell extracts of the mutant (NC1) and parent were separated electrophoretically in nondenaturing polyacrylamide gels and stained for catalase activity, strain NC1 was found to lack a functional HPII that is normally produced by the parental strain (Fig. 1, lanes A and B). To overcome the problems associated with multiple prophage (2), the fusion was transduced into a *rec*$^+$ *lac* deletion strain (GC4468) (Fig. 1, lanes C and D).

Since two loci were identified which affected HPII activity, it was necessary to map the mutation by cotransduction of nearby markers. The mutation was not cotransducible with markers close to either *katF* at 59 min (i.e., *zhf*::Tn*10*) or *katG* at 88 min (i.e., *sodA*::MPR13) (data not shown).

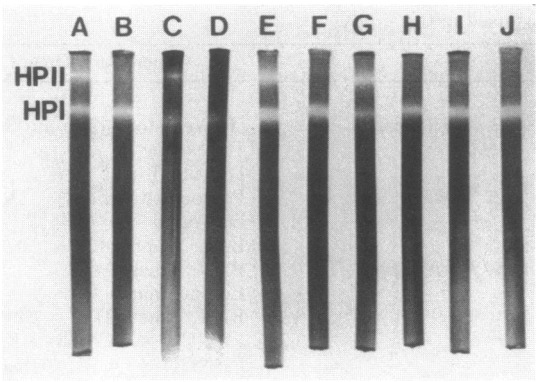

FIG. 1. Catalase isozymes. Cell extracts were prepared, separated electrophoretically, and stained as described in the text. Samples containing 100 μg of protein were separated electrophoretically for 3 h at 4 mA per tube. Lanes: A, SE5000; B, NC1; C, GC4468; D, NC4468; E, RK4936; F, NC4936; G, TA4112; H, NC4112; I, QC781; J, DTAK.

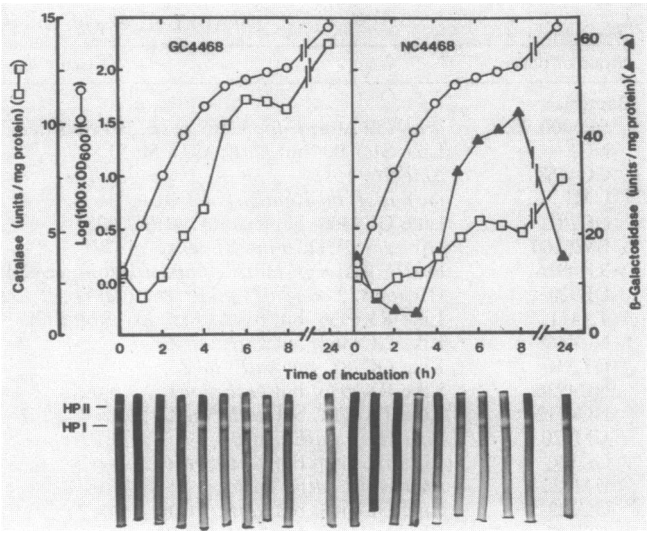

FIG. 2. Catalase and β-galactosidase expression under anaerobic conditions. Overnight cultures of strains GC4468 and NC4468 grown on LB broth–0.5% glucose were inoculated into 200-ml volumes of the same medium to an initial OD_{600} of 0.010. Cell extracts were prepared and assayed for catalase, β-galactosidase, and protein as described in the text. Fractions containing 100 μg of protein were applied to the gels, separated electrophoretically, and stained for catalase isozymes as described in the text. The gels appearing below each sampling time show the two isozymes (HPI and HPII) that were produced at each point. Note the induction of HPII in GC4468 during the late exponential phase.

However, it was found to be approximatey 35% cotransducible with *zdh*::Tn*10* (Table 2). Since mutations in this locus have been described previously (14), we compared the cotransduction frequency of *katE*::*lacZ* with *zdh*::Tn*10* with that of well-characterized *katE* mutations.

Although UM1 is deficient in both catalases, we found that *katE*$^+$ transductants of this strain could easily be scored for oxygen evolution from hydrogen peroxide by using the plate assay described above. The results (Table 2) indicate that *katE* in UM1 and the mutation in NC4468 exhibit similar cotransduction frequencies with the *zdh*::Tn*10* marker. A simple 2 by 2 chi-square analysis revealed no significant difference in the frequency of catalase-positive transductants that were obtained ($P > 0.10$). Similarly, the *katE* mutation in UM1 and NC4468 were both found to be 70% linked to *pfkB* (data not shown).

When *katE*::Tn*10* was introduced into NC4468 by generalized transduction, fewer than 0.7% β-galactosidase-positive recombinants were observed among the tetracycline-resistant transductants (Table 2), indicating that these two mutations are closely linked. These transductional data in toto suggest that the *kat*::*lacZ* fusion is in *katE*, a locus that affects the synthesis of HPII. The various reported linkages of different *katE* mutations with known markers in the 38′ region (14) may indicate that more than one gene in this part of the genome is involved in HPII expression. Thus, a more definite localization of the *katE*::*lacZ* fusion isolated in this study must await complementation analyses. As yet, the *katE* gene(s) of *E. coli* has not been isolated to permit such tests.

When *katF*::Tn*10* was introduced into strain NC4468, β-galactosidase activity was substantially reduced in transductant colonies that were selected on LB plates containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside. This suggested a possible regulatory role for *katF* in *katE* expression, a hypothesis which was subsequently explored more fully (see below). As expected, control transductions of *katF*::Tn*10* into GC4468 yielded only catalase-negative colonies (data not shown).

TABLE 2. Transductional analysis of *kat*::*lacZ*[a]

Donor strain	Recipient strain	Unselected marker	No. (% of total)
BW6160 (*zdh*::Tn*10*)	UM1 (*katE*)	Kat$^+$	76 (42)
		Kat$^-$	107 (58)
BW6160 (*zdh*::Tn*10*)	NC4468 (*kat*)	Kan^s	59 (35)
		Kan^r	110 (65)
UM120 (*katE*::Tn*10*)	NC4468 (*kat*)	Lac$^-$	636 (99.4)
		Lac$^+$	4 (0.6)

[a] The selected marker for all strains was Tet^r.

Relationship between catalase and *katE*::*lacZ* expression. (i) Anaerobic conditions. Cultures of strains GC4468 and NC4468 were grown anaerobically in LB broth containing 0.5% glucose. Cell extracts were prepared and separated electrophoretically to correlate quantitative changes in catalase and β-galactosidase activities with the appearance of the two catalase species. The HPII protein was found to be synthesized by GC4468 under anaerobic conditions and could be correlated to increases in β-galactosidase activity in NC4468 (Fig. 2). The data indicate that both enzyme activities were induced as the cultures entered the stationary phase, in a pattern reminiscent of the aerobic expression of HPII (Fig. 3 of reference 15), although the absolute levels of activity obtained (Fig. 2) were three- to fourfold lower. The expression of *katE* under anaerobic conditions was a surprising result since HPII is not known to be produced in anaerobically grown cells (11).

Catalase activity in anaerobically grown NC4468 (representing HPI synthesis) was also found to increase slightly as the culture entered the stationary phase of growth. This increase in catalase activity could be correlated to an increase in the intensity of the HPI band observed over time in both GC4468 and NC4468 (Fig. 2).

(ii) Aerobic conditions. The kinetics of enzyme synthesis were examined in cells that were grown aerobically in LB medium (Fig. 3). Since the inocula used in these experiments were obtained from late-exponential-phase cultures, they possessed relatively high levels of catalase in the case of

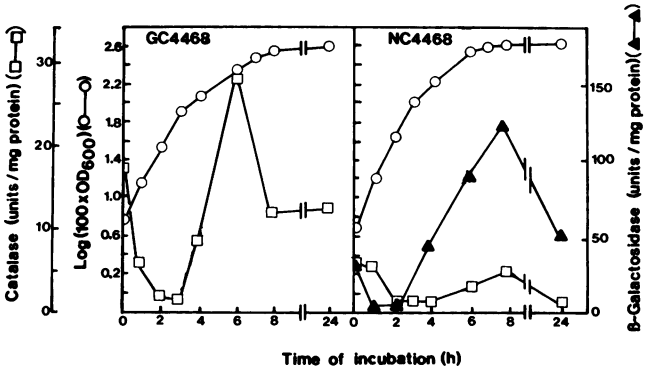

FIG. 3. Aerobic expression of catalase and β-galactosidase. Exponentially growing cultures of strains GC4468 and NC4468 growing in LB broth were inoculated into 200-ml volumes of the same medium to an initial OD_{600} of 0.05. Periodically, samples were removed and assayed for catalase and β-galactosidase activities as described in the text.

strain GC4468 and β-galactosidase in the case of strain NC4468. The observed reduction in specific catalase activity as the cultures grew in fresh broth could, however, be accounted for by a dilution of preexisting enzyme by the growing culture. The same was found to be true of β-galactosidase synthesis. These data indicate that *katE*::*lacZ* expression (or HPII synthesis for that matter) is negligible during the early exponential phase of growth.

(iii) Response to hydrogen peroxide. Catalases are induced by hydrogen peroxide (15, 22), and they confer protection to the cell from both near-UV light (8, 27) and hydrogen peroxide (12, 13). Recently, catalase (HPI) induction by hydrogen peroxide in *E. coli* was shown to be regulated by *oxyR* (3, 22). To further examine the response of catalase and *katE*::*lacZ* to hydrogen peroxide and the effect of the oxidative stress regulon on the synthesis of HPII, the fusion was transduced into a strain deleted for *oxyR*, a gene that encodes the positive regulatory protein for a hydrogen peroxide-inducible regulon (Fig. 1, lanes E, F, G, and H).

Catalase activity increased fourfold in exponential-phase $oxyR^+$ strains (RK4936 and NC4936) that were exposed to 60 μM hydrogen peroxide for 60 min but not in the *oxyR* deletion strains (TA4112 and NC4112) (Table 3). The 50% increase in catalase activity in TA4112 and NC4112 during hydrogen peroxide exposure (Table 3) was similar to that observed in untreated control cultures that were incubated in parallel. *katE*::*lacZ* expression was found to be unaffected by hydrogen peroxide, but it was expressed at twofold higher levels in NC4112 (deleted for *oxyR*) than NC4936 ($oxyR^+$). These results confirm that only HPI is subject to regulation by hydrogen peroxide and is dependent on the OxyR protein.

(iv) Parallel HPII and *katE*::*lacZ* expression. The results shown in Fig. 2 and 3 strongly suggest that the HPII protein and *katE*::*lacZ* are expressed under the same conditions. However, since strain GC4468 possesses both catalases (HPI and HPII), catalase activity in this strain cannot be directly compared with β-galactosidase activity in strain NC4468. To overcome this problem, a *katG*::Tn*10* mutation was transduced into both GC4468 and NC4468 to abolish HPI synthesis. The expression of catalase and β-galactosidase was measured in the resulting strains (GC202 and NC202) to determine whether HPII and *katE*::*lacZ* were expressed in parallel. Parallel expression was observed in exponential-phase cultures growing in both rich and minimal media (Fig. 4).

(v) Response to other forms of oxidative stress. Neither catalase nor β-galactosidase activity was affected when cells grown on minimal medium at 28°C were shifted at 42°C, a thermal stress that is sufficient to induce the heat shock response (7) (data not shown).

Manganese superoxide dismutase (10) and HPII (11, 15; this study) are both induced by aeration and together catalyze the ultimate dismutation of O_2^- to water and oxygen. Therefore, *katE*::*lacZ* was introduced into a *sodA* mutant strain (deficient in manganese superoxide dismutase synthesis) to determine whether *katE* expression was altered. No difference in either catalase or β-galactosidase activities were observed in these strains (data not shown).

Induction of *katE*::*lacZ* by late-exponential-phase culture supernatant. Since *katE* expression appeared to be a function of the growth phase, we tested whether expression could be induced by suspending early-exponential-phase cells (uninduced) in the supernatant from late-exponential-phase cultures. If the inducing agent was extracellular rather than intracellular, one would expect *katE*::*lacZ* (and HPII) to be induced. To test this hypothesis, fractions of supernatant were collected from a culture of *E. coli* throughout the exponential and early stationary phases. The ability of these supernatants to cause the induction of *katE* and HPII in early-exponential-phase cells was assessed as described in

TABLE 3. Effect of *oxyR* on the expression of catalase and β-galactosidase in *oxyR* deletion and parental strains harboring *katE*::*lacZ* fusions[a]

Strain	Relevant genotype	Sp act (U/mg)			
		Catalase		β-Galactosidase	
		Initial[b]	With 60 μM H_2O_2[c]	Initial[b]	With 60 μM H_2O_2[c]
RK4936	$oxyR^+$	23.2	75.6 ± 6.2	ND[d]	ND
TA4112	*oxyRΔ3*	21.4	29.9 ± 3.4	ND	ND
NC4936	$oxyR^+$ *katE*::*lacZ*	14.0	56.5 ± 9.2	56	54 ± 4
NC4112	*oxyR Δ3* katE::*lacZ*	10.3	17.3 ± 0.3	111	103 ± 4

[a] Overnight cultures grown on GYA medium were inoculated into prewarmed GYA medium to an initial OD_{600} of 0.02. When the cultures reached an OD_{600} of 0.3, 5.0-ml volumes were placed into flasks with hydrogen peroxide to yield a final treatment concentration of 60 μM. Control cultures were incubated in parallel in the absence of added hydrogen peroxide. After 60 min of incubation at 37°C, chloramphenicol (150 μg/ml) was added to stop further protein synthesis. Samples were immediately assayed for OD_{600} and catalase and β-galactosidase activities.

[b] Specific activity immediately prior to incubation in the presence of hydrogen peroxide.

[c] Values are means ± SE standard error (incubations were performed in triplicate).

[d] ND, Not determined.

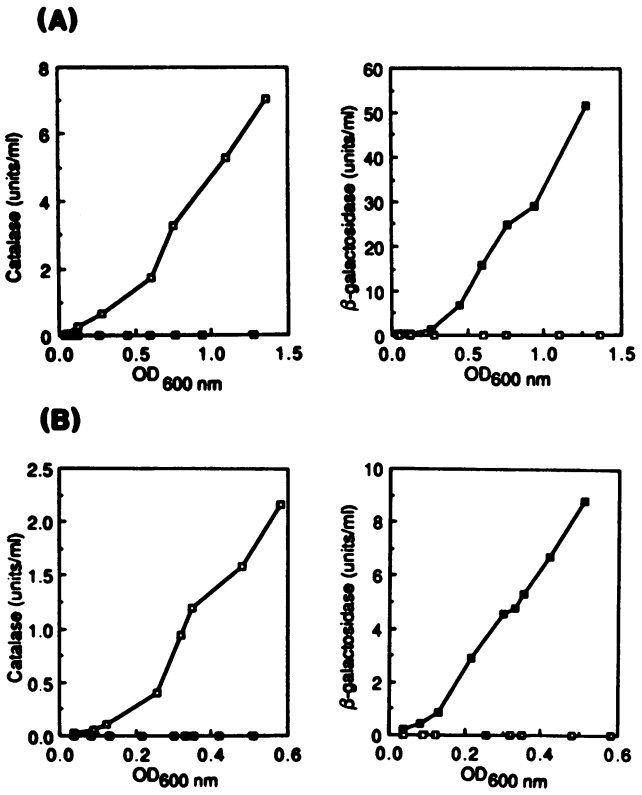

FIG. 4. Parallel expression of *katE* and HPII in rich and minimal media. Mid-exponential-phase cultures of strains GC202 and NC202 growing in LB medium–100 mM PIPES (pH 7.0) and GMM were subcultured into the same media to an initial OD_{600} of 0.05. Samples were periodically removed and assayed for OD_{600} and catalase and β-galactosidase activities, as indicated. (A) LB–100 mM PIPES; (B) GMM. Symbols: □, GC202; ■, NC202.

footnote *a* of Table 3. Results indicated that both *katE* and HPII expression are induced by the supernatant derived from late-exponential-phase cultures but not the early-exponential-phase cultures (Table 4). As expected, no induction was observed in control medium incubations. These results suggest that the inducing regimen accumulates in the extracellular phase during growth and reaches a maximum at the end of logarithmic growth.

Effect of *katF* on *katE* expression. Since *katF* and *katE* are both required for HPII synthesis, it is possible that one locus performs a regulatory role. We tested whether a lesion in *katF* would affect *katE* expression by introducing a *katF*::Tn*10* mutation of strain UM122 into strain NC4468 (*katE*::*lacZ*). The resulting fusion strain, NC122, produced low basal levels of β-galactosidase (<5.0 U/mg of protein) which were not induced to higher levels when the culture was allowed to grow into the stationary phase or when early-exponential-phase cells of NC122 were suspended in the culture supernatant from a late-exponential-phase culture of strain NC4468 (Fig. 5).

DISCUSSION

Loewen and Triggs (16) have previously shown that both *katE* and *katF* are required for HPII synthesis. While the relationship between the two loci has been the subject of speculation (24), little discriminating experimental evidence has appeared. One possibility is that one locus is a structural

TABLE 4. Induction of HPII catalase and *katE* by late-exponential-phase culture supernatants[a]

Type of supernatant	OD_{600} of culture[b]	Sp act (U/mg)	
		Catalase[c]	β-Galactosidase[d]
Control (LB broth)	0.0	ND[e]	3.1
Mid-exponential phase	0.17	ND	7.0
	0.35	3.2	12.6
Late exponential phase	0.72	8.4	21.9
	1.25	7.3	47.2
Early stationary phase	1.55	ND	14.9
	1.88	ND	16.4

[a] Strain GC202 was grown aerobically in 200 ml of LB medium at 37°C and 200 rpm. Periodically (every 30 min), 20-ml samples were taken, the cells removed by centrifugation, and the culture supernatant was stored in ice for later use. The ability of these supernatants to induce HPII and *katE* in naive (uninduced) cultures was tested by incubating washed, early-exponential-phase cells (OD_{600} of 0.100) in an equal volume of supernatant, as indicated. These suspended cells were incubated at 37°C with shaking (200 rpm) at 37°C for 60 min, after which the cells were collected by centrifugation and assayed for β-galactosidase (NC202) and catalase (GC202) activities.

[b] The OD_{600} of the culture at the time of supernatant collection.

[c] Assayed with strain GC202 (*katG*::Tn*10* *katE*$^+$).

[d] Assayed with strain NC202 (*katG*::Tn*10* *katE*::*lacZ*).

[e] ND, Not detected.

gene and that the other is a regulatory gene, since HPII is a tetramer of similarly sized subunits and a mutation in either gene eliminates HPII function (14, 16), precluding the possibility of duplicate genes. Alternatively, each locus could encode a single subunit which must associate together to form an active tetrameric enzyme. The data from this study, indicating that *katE* expression parallels that of the HPII catalase in exponentially growing cultures, support a structural role for this locus. Since a functional *katF* gene is necessary for *katE* expression (this study), it is likely that

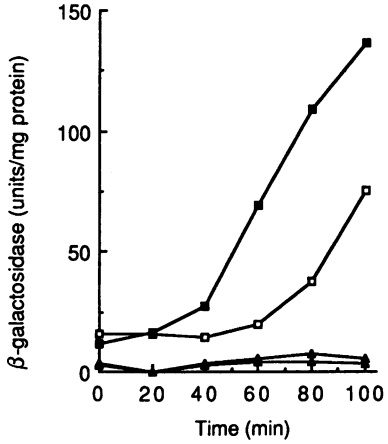

FIG. 5. Effect of *katF* on *katE* expression. Overnight cultures of strains NC4468 and NC122 in LB medium–100 mM PIPES (pH 7.0) were subcultured into 50 ml of the same medium to an initial OD_{600} of 0.010. When the cultures reached an OD_{600} of 0.3, they were divided into two 25-ml portions, centrifuged (6,000 × *g* for 10 min at 22°C), and suspended in either fresh prewarmed medium or clarified supernatant from a late-exponential-phase culture (OD_{600} of 1.2) of NC4468, as indicated. These cultures were then incubated at 200 rpm (Lab-Line orbital shaker) and 37°C for 100 min. Samples (1.7 ml) were removed periodically, washed by centrifugation in a microfuge, and assayed for β-galactosidase activity. Symbols: □, NC4468 suspended in fresh medium; ■, NC4468 suspended in clarified supernatant; △, NC122 suspended in fresh medium; ▲, NC122 suspended in clarified supernatant.

katF plays a regulatory role. The *katE* and *katF* genes are noncontiguous loci (14, 16), and the observed effects of *katF* on *katE* expression are therefore *trans* in nature. A conservative interpretation of these observations would be that *katF* is a *trans*-acting, positive regulator of *katE* expression. As a possible regulatory element, *katF* may have an additional role(s) in the stress response. The fact that *katF*, but not *katE*, mutants are hypersensitive to near UV (24) may, in part, be a reflection of this.

Studies on the regulation of catalase synthesis in *E. coli* are complicated by the fact that the two species produced differ in their biochemical properties (5) and regulation (15). Thus, while it has long been known that catalase activity is induced in stationary-phase cultures (9, 11), by aeration (11, 30), or by hydrogen peroxide (3, 15), the identity of the species induced by each of these regimens has only recently been revealed by using mutants that were deficient in the synthesis of each of the catalase species (15, 19). Regulatory studies are further complicated by the fact that although hydrogen peroxide is decomposed by both enzymes, it serves as an inducer of HPI only. Presumably, a deficiency in HPII synthesis could conceivably lead to higher concentrations of intracellular hydrogen peroxide which could, in turn, induce HPI to higher levels than would be found in a strain that was proficient in HPII synthesis.

Other investigators have shown that the induction of catalase by hydrogen peroxide is *oxyR* dependent (3) and have demonstrated that the synthesis of HPI is regulated by *oxyR* at the transcriptional level (22). The results of this study confirm that HPI is induced by hydrogen peroxide in an *oxyR*-dependent mechanism either in the presence or absence of a functional HPII protein. The data indicate that *katE* (HPII) is not part of the *oxyR* regulon. Furthermore, neither the synthesis of HPII nor the transcription of *katE* was found to be affected by the presence of hydrogen peroxide.

The finding that *katE* is expressed under anaerobic conditions led us to reexamine catalase synthesis in *E. coli* under anaerobic conditions. HPII, which was previously thought to be produced only under aerobic conditions (11), was also detected in cells that were grown anaerobically and was induced as the cells entered the stationary phase. The metabolic need for catalase under these conditions is not clear, since hydrogen peroxide cannot be generated in the absence of oxygen. However, since HPII levels are essentially independent of the presence of hydrogen peroxide (15; this study), this protein may serve some other unknown function, perhaps in protecting the cell during transient exposure to oxygen.

HPII synthesis and transcriptional regulation of *katE* expression are a function of the growth phase of the cell. The metabolic signal for the rapid increase in HPII levels at the end of the exponential phase of growth is not yet known. At this point the bacterial cell is undergoing changes in metabolism which result in higher respiratory activity and an increase in the accumulation of tricarboxylic acid cycle intermediates. Both of these events have been correlated with an increase in catalase synthesis in *E. coli* (11, 15). Although the point of maximal catalase synthesis also coincides with low pH (9, 11), cultures of *Salmonella typhimurium* in which the pH was maintained at neutrality still exhibited induction of catalase at the end of the exponential phase of growth (9). Similarly, we found that catalase induction in *E. coli* occurs in LB medium that was buffered to minimize pH fluctuation during growth (this study). The finding that *katE* expression was induced by suspending noninduced, early-exponential-phase cells in conditioned supernatant taken from an early-stationary-phase culture strongly suggests that the signal required for induction accumulates extracellularly. However, attempts to identify a metabolic product which accumulates in the extracellular phase and acts as an inducer of *katE* expression have not, as yet, been successful.

Since high levels of HPII are found in cells which are grown on weak acids such as lactate, acetate, and tricarboxylic acid cycle intermediates (9, 15, 30) and under anaerobic conditions (this study), the regulation of this catalase must be more complex than previously thought. Recently, it has been shown that members of an acid-sensitive regulon in *E. coli* with an unknown function are induced in the presence of weak acids such as sodium benzoate or sodium salicylate in a pH-independent fashion (26), presumably by a lowering of the intracellular pH. Such control could account for the observed pH-independent regulation of HPII synthesis in *E. coli* and *S. typhimurium*, particularly the anaerobic expression of HPII noted in this study. Preliminary results indicate that nonmetabolic weak acids can act as inducers of *katE::lacZ* expression.

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LITERATURE CITED

1. **Beers, R. F., and I. W. Sizer.** 1952. A spectrophotometric assay for measuring the breakdown of hydrogen peroxide by catalase. J. Biol. Chem. **196**:133–140.
2. **Bremer, E., T. J. Silhavy, and G. M. Weinstock.** 1985. Transposable λ*plac*Mu bacteriophages for creating *lacZ* operon fusions and kanamycin resistant insertions in *Escherichia coli*. J. Bacteriol. **160**:595–601.
3. **Christman, M. F., R. W. Morgan, F. S. Jacobson, and B. N. Ames.** 1985. Positive control of a regulon for defenses against oxidative stress and some heat-shock proteins in *Salmonella typhimurium*. Cell **41**:753–762.
4. **Claiborne, A., and I. Fridovich.** 1979. Purification of the orthodianisidine peroxidase from *Escherichia coli* B. J. Biol. Chem. **254**:4245–4252.
5. **Claiborne, A., D. P. Malinowski, and I. Fridovich.** 1985. Purification and characterization of hydroperoxidase II of *Escherichia coli* B. J. Biol. Chem. **254**:11664–11668.
6. **Clare, D. A., M. N. Duong, D. Darr, F. Archibald, and I. Fridovich.** 1984. Effects of molecular oxygen on the detection of superoxide radical with nitroblue tetrazolium and an activity strain for catalase. Anal. Biochem. **140**:532–537.
7. **Cowing, D. W., J. C. A. Bardwell, E. A. Craig, C. Woolford, R. W. Hendrix, and C. A. Gross.** 1985. Consensus sequence for *Escherichia coli* heat shock promoters. Proc. Natl. Acad. Sci. USA **82**:2679–2683.
8. **Eisenstark, A., and G. Perot.** 1987. Catalase has only a minor role in protection against near-UV radiation damage in bacteria. Mol. Gen. Genet. **207**:68–72.
9. **Finn, G. J., and S. Condon.** 1975. Regulation of catalase synthesis in *Salmonella typhimurium*. J. Bacteriol. **123**:570–579.
10. **Hassan, H. M., and I. Fridovich.** 1977. Enzymatic defenses against the toxicity of oxygen and streptonigrin in *Escherichia coli*. J. Bacteriol. **129**:1574–1583.
11. **Hassan, H. M., and I. Fridovich.** 1978. Regulation of the synthesis of catalase and peroxidase in *Escherichia coli*. J. Biol. Chem. **253**:6445–6450.
12. **Imlay, J. A., and S. Linn.** 1987. Mutagenesis and stress responses induced in *Escherichia coli* by hydrogen peroxide. J. Bacteriol. **169**:2967–2976.

13. **Linn, S., and J. A. Imlay.** 1987. Toxicity, mutagenesis and stress responses induced in *Escherichia coli* by hydrogen peroxide. J. Cell. Sci. **6** (Suppl.):289–301.
14. **Loewen, P. C.** 1984. Isolation of catalase-deficient *Escherichia coli* mutants and genetic mapping of *katE*, a locus that affects catalase activity. J. Bacteriol. **157**:622–626.
15. **Loewen, P. C., J. Switala, and B. L. Triggs-Raine.** 1985. Catalases HPI and HPII in *Escherichia coli* are induced independently. Arch. Biochem. Biophys. **243**:144–149.
16. **Loewen, P. C., and B. L. Triggs.** 1984. Genetic mapping of *katF*, a locus that with *katE* affects the synthesis of a second catalase species in *Escherichia coli*. J. Bacteriol. **160**:668–675.
17. **Loewen, P. C., B. L. Triggs, C. S. George, and B. Hrabarchuk.** 1985. Genetic mapping of *katG*, a locus that affects synthesis of the bifunctional catalase-peroxidase hydroperoxidase I in *Escherichia coli*. J. Bacteriol. **162**:661–667.
18. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
19. **Meir, E., and E. Yagil.** 1984. Catalase-negative mutants of *Escherichia coli*. Curr. Microbiol. **11**:13–18.
20. **Miller, J.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. **Moody, C. S., and H. M. Hassan.** 1984. Anaerobic biosynthesis of the manganese-containing superoxide dismutase in *Escherichia coli*. J. Biol. Chem. **259**:12821–12825.
22. **Morgan, R. W., M. F. Christman, F. S. Jacobson, G. Storz, and B. N. Ames.** 1986. Hydrogen peroxide-inducible proteins in *Salmonella typhimurium* overlap with heat shock and other stress proteins. Proc. Natl. Acad. Sci. USA **83**:8059–8063.
23. **Poole, R. K., B. S. Baines, and C. A. Appelby.** 1986. Haemoprotein b-590-a reducible catalase and peroxidase: evidence for its close relationship to hydroperoxidase I and 'a cytochrome a_1b' preparation. J. Gen. Microbiol. **132**:1525–1539.
24. **Sammartano, L. J., R. W. Tuveson, and R. Davenport.** 1986. Control of sensitivity to inactivation by H_2O_2 and broad spectrum near-UV radiation by the *Escherichia coli katF* locus. J. Bacteriol. **168**:13–21.
25. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
26. **Solnczewski, J. L., T. N. Gonzalez, F. M. Bartholomew, and N. J. Holt.** 1987. Mu d-directed *lacZ* fusions regulated by low pH in *Escherichia coli*. J. Bacteriol. **169**:3001–3006.
27. **Tuveson, R. W.** 1980. The interaction of a gene (*nur*) controlling near UV sensitivity and the *polA1* gene in strains of *Escherichia coli*. Photochem. Photobiol. **33**:919–923.
28. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97–106.
29. **Wanner, B. L.** 1986. Novel regulatory mutants of the phosphate regulon in *Escherichia coli*. J. Mol. Biol. **191**:39–58.
30. **Yoshpe-Purer, Y., Y. Henis, and J. Yashphe.** 1977. Regulation of catalase level in *Escherichia coli*. Can. J. Microbiol. **23**:84–91.