JOURNAL OF BACTERIOLOGY, Mar. 1994, p. 1297–1302
0021-9193/94/$04.00+0

Vol. 176, No. 3

The Δ(*argF-lacZ*)*205*(U169) Deletion Greatly Enhances Resistance to Hydrogen Peroxide in Stationary-Phase *Escherichia coli*

MICHAEL R. VOLKERT,[1]* PETER C. LOEWEN,[2] JACEK SWITALA,[2] DAVID CROWLEY,[1] AND MATTHEW CONLEY[1]

Department of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester, Massachusetts 01655,[1] and Department of Microbiology, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada[2]

Received 23 September 1993/Accepted 17 December 1993

In this study, we demonstrate that a strain bearing the Δ(*argF-lacZ*)*205*(U169) deletion exhibits a high level of resistance to hydrogen peroxide compared with its undeleted parent. Our initial investigation of the mechanism behind the observed differences in peroxide resistance when parent and mutant strains are compared indicates that the parent strain carries a region near *argF* that is responsible for the H_2O_2-sensitive phenotype, which we have named *katC*. The H_2O_2 resistance phenotype of the Δ*katC* [Δ(*argF-lacZ*)*205*(U169)] mutant strain can be duplicated by Tn*9* insertion in a specific locus (*katC5*::Tn*9*) which maps near *argF*. The increased H_2O_2 resistance of the Δ*katC* and *katC5*::Tn*9* mutant strains can be seen only when cells are grown to stationary phase; exponential-phase cells are unaffected by the presence or absence of *katC*. This H_2O_2 resistance mechanism requires functional *katE* and *katF* genes, which suggests that the mechanism of H_2O_2 resistance may involve the activity of the stationary-phase-specific catalase HPII. Cloning, DNA sequencing, and analysis of the *katC5*::Tn*9* insertion allele in comparison with its parent allele implicate two insertion elements, IS*1*B and IS*30*B, and suggest that their presence sensitizes parent cells to H_2O_2.

All aerobically growing, respiring cells suffer from the deleterious effects of oxidative metabolism. These effects result from the oxidation of various cellular components such as DNA, RNA, proteins, and lipids (5, 9, 20). To cope with oxidative damage, cells have evolved a complex network of genes comprising several different global regulons. Many of the genes that comprise the oxidative defense systems are regulated and are induced when cells encounter elevated levels of oxidative compounds such as superoxide, hydrogen peroxide, and organic peroxides. The genes induced by these agents include genes whose products detoxify these reactive compounds and genes whose products repair the DNA damage that they produce (7, 11).

Escherichia coli expresses different sets of oxidation protective genes at different stages of its life cycle. During active growth, the genes induced by oxidative agents that perform these functions are the genes comprising the *soxRS* and *oxyR* regulons (7, 11). As cells enter stationary phase, genes encoding a new catalase, an AP endonuclease, and perhaps other genes that either protect or repair DNA are induced and expressed at high levels even without oxidative treatments (1, 4, 13, 14, 23, 24, 26, 28, 29). The stationary-phase peroxide resistance genes require the *katF*-encoded σ^S for their transcription (21, 24, 26, 27, 36). Examination of induced proteins by two-dimensional polyacrylamide gel electrophoresis indicates that there are many additional proteins induced by oxidative damage, but their regulatory mechanisms have not yet been identified, much less characterized (12, 37).

In this study, we observed that a strain carrying the chromosomal region between *argF* and *lacZ* is considerably more sensitive to H_2O_2 than a strain with the *argF-lacZ* region deleted, suggesting that the *argF-lacZ* region carries a gene, or genes, that sensitizes cells to H_2O_2. A gene that sensitizes the cell to H_2O_2 seems unusual in the face of so many protective responses, and we have therefore initiated a study to determine the genetic and biochemical nature of this phenomenon. This report describes the identification and initial characterization of a locus near 6 min on the genetic map that significantly enhances the H_2O_2 sensitivity of *E. coli*. This locus is in the same region as the *Salmonella typhimurium katC* gene, which affects H_2O_2 sensitivity but has never been characterized. We have named the *E. coli* H_2O_2 sensitivity gene *katC*, in keeping with the *S. typhimurium* nomenclature.

MATERIALS AND METHODS

Bacterial strains, media, and genetic methods. All bacterial strains used in this study are listed in Table 1. Survival studies were performed with cells grown in LB broth (34), and survival assays were done with LB agar plates. When plasmid-bearing strains or strains carrying Tn*9* were analyzed, ampicillin (100 μg/ml) or chloramphenicol (50 μg/ml) was added. Medium for β-galactosidase assays was our standard E minimal medium (34). λ broth and λ plates were used for λ survival assays (1% tryptone, 0.25% NaCl; for plates, 1.1% agar was added; for soft agar overlays, 0.6% agar was added).

H_2O_2 survival assays. Cells were grown overnight (minimum of 20 h) in LB broth with aeration at 37°C, washed, and resuspended in cold E salts. Three milliliters of this cell suspension was diluted in E salts to a final volume of 10 ml, from which 9 ml was treated with 1 ml of H_2O_2 freshly diluted to the appropriate concentration. After the addition of H_2O_2, samples were removed at different times, immediately diluted, and plated on LB plates or LB plates containing the appropriate antibiotics. In the case of λ survival experiments, λ phage stocks were treated with H_2O_2, then diluted in 0.1 M Mg salts, and used to infect overnight cultures prepared as described above except that cells were grown and resuspended in λ broth. After infection, 3 ml of λ soft agar was added, and cells were plated on λ plates and incubated overnight at 37°C.

Enzyme assays. Catalase activity was determined as previ-

* Corresponding author.

TABLE 1. Bacterial strains used

Strain[a]	Genotype	Reference or source
MV1161	*thr-1 ara-14 leuB6* Δ(*gpt-proA*)*62 lacY1 tsx-33 supE44 galK2 hisG4 rfbD1 mgl-51 rpsL31 kdgK51 xyl-5 mtl-1 argE3 thi-1 rfa-550*	35
MV2640	As MV1161 Δ(*argF-lacZ*)*205*(U169) (also Δ*katC*) *pro*$^+$	This work
MV2689	As MV1161 *katC5*::Tn*9*	This work
MV2909	As MV1161 Δ(*argF-lacZ*)*205*(U169) *pro*$^+$ F′ *lacZ*::Tn*10 proAB*$^+$	This work
MV2920	HfrKL226 (PO2A)/pMV106	17
MV2922	As MV1161 *katE12*::Tn*10*	This work
MV2923	As MV1161 *katE12*::Tn*10* Δ(*argF-lacZ*)*205*(U169)	This work
MV2974	As MV1161 λRS*katE16*Φ*lacZ* Δ(*argF-lacZ*)*205*(U169)	This work
MV3206	As MV1161 λRS*katE16*Φ*lacZ*Δ (*argF-lacZ*)*205*(U169)/F′ *lacZ*::Tn*10 proAB*$^+$	This work

[a] MV1161 is a spontaneous *rfa-550* (φX174s) derivative of AB1157. MV2640 was produced by mating HfrH (Δ(*argF-lacZ*)*205*(U169)) with MV1161, selecting for Pro$^+$ recombinants, and then screening for coinheritance of the Lac$^-$ allele, indicating the presence of the Δ(*argF-lacZ*)*205*(U169) (also Δ*katC*) mutation. Construction of MV2689 is described in the text. MV2909 was constructed by introducing the F factor F′ *lacZ*::Tn*10 proAB*$^+$ into strain MV2640 by mating. MV2920 is a pMV106 transformant of KL226 (HfrPO2A) and carries the following additional markers: *relA1 tonA22* T2^R *pit-10 spoT1*. MV2922 is a *katE12*::Tn*10* transductant of MV1161 produced by using P1-UM120 (*katE12*::Tn*10*) (16). MV2923 is a *katE12*::Tn*10* transductant of MV2640 produced by using P1-UM120 (*katE12*::Tn*10*) (16). MV2974 is a λRS*katE16* lysogen of MV2640. MV3206 is a derivative of MV2974 which carries F′ *lacZ*::Tn*10 proAB*$^+$ *katC*$^+$.

ously described (15). β-Galactosidase activity was determined as previously described (34).

Tn*9* insertion mutagenesis. To produce Tn*9* insertions targeted to the *argF*-to-*lac* region of the chromosome, a mating-out procedure was used. The chloramphenicol-resistant (Cm^r) Tn*9*-containing nontransmissible plasmid pMV106 (33) was first introduced into KL226, an Hfr strain that transfers *lac*$^+$ as an early marker, to construct MV2920 (17). MV2920 was then used as a donor in a mating with strain MV2640, selecting for Lac$^+$ Cm^r. Such recombinants can form only by incorporating the entire region from *lac* through *argF* and therefore inherit the *katC*$^+$ allele and become H_2O_2 sensitive unless Tn*9* has inactivated *katC* by insertion. The Cm^r phenotype of Tn*9* is transferred as a result of transposition to the chromosome, or as a cointegrate which rapidly resolves, producing free plasmid DNA (6, 33). In either case, Tn*9* is inserted into the region of the chromosome that is transferred, and insertions into the desired region are highly enriched. Strains carrying plasmid pMV106 DNA were eliminated by screening for the presence of plasmid DNA by gel analysis. Strains carrying plasmids as cointegrates were identified by Southern hybridizations, using the vector regions of the nontransmissible plasmid as a probe to detect plasmid DNA integrated into the chromosome. H_2O_2-resistant insertion mutants were identified by growing cells overnight in LB and then spotting cells on LB plates containing different concentrations of H_2O_2 (0, 20, and 30 mM). After preliminary testing, standard survival tests were performed as described above to identify true H_2O_2-resistant Tn*9* insertion mutants. To confirm that the insertions were responsible for the H_2O_2 resistance phenotype, the presumptive *katC*::Tn*9* insertion mutations were crossed into the wild-type strain, MV1161, by P1 transduction, and the Cm^r recombinants were tested for coinheritance of the H_2O_2 resistance phenotype.

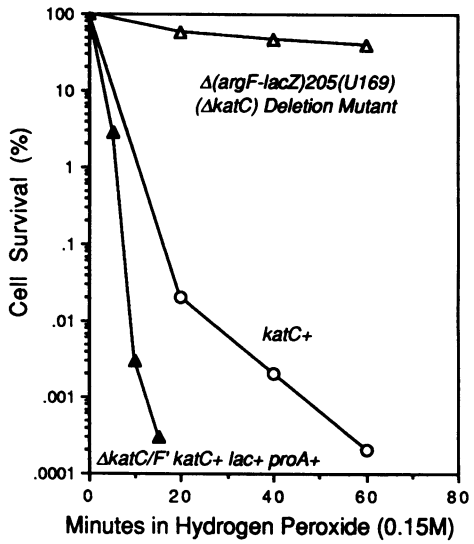

FIG. 1. Effects of the Δ(*argF-lacZ*)*205*(U169) deletion with and without the F′ *lac*$^+$ *pro*$^+$ plasmid on H_2O_2 sensitivity in cells grown to stationary phase. The strains studied include MV1161 (parent) (○), MV2640 [Δ(*argF-lacZ*)*205*(U169)] (△), and MV2909 [Δ(*argF-lacZ*)*205*(U169)/F′ *lac*$^+$ *pro*$^+$] (▲).

Cloning and DNA sequencing. Cloning of *katC*$^+$ and *katC5*::Tn*9* alleles was performed by standard methods (19), using pBluescript SK+ (Stratagene) as a vector. For cloning of the *katC5*::Tn*9* mutant allele, clones that confer chloramphenicol resistance were selected after DNA purification, restriction enzyme digestion, and ligation to pBluescript by plating transformants on LB plates containing chloramphenicol. DNA flanking the Tn*9* insertion was used as a hybridization probe in subsequent experiments to identify clones carrying the wild-type region corresponding to the *katC5*::Tn*9* region.

Nucleotide sequence accession number. The *katC* sequence can be obtained under GenBank accession number L20943.

RESULTS

Peroxide resistance of the Δ(*argF-lacZ*)*205*(U169) deletion mutant. The results in Fig. 1 demonstrate that strain MV2640, which bears the common deletion Δ(*argF-lacZ*)*205*(U169), is considerably more resistant to H_2O_2 than its parent, MV1161. The H_2O_2 resistance phenotype can be complemented by a standard F′ *lac*$^+$ *pro*$^+$ plasmid (Fig. 1) which restores H_2O_2 sensitivity. Thus, the wild-type allele is dominant, as would be expected for a deletion mutation. Because complementation of the deletion mutant sensitizes the cells to H_2O_2, the *lac-argF* region of the chromosome carries a gene, or genes, that causes sensitivity to H_2O_2. We have named the H_2O_2 sensitization gene *katC* and will refer to the Δ(*argF-lacZ*)*205*(U169) deletion mutation as Δ*katC*.

Δ*katC* does not enhance repair of H_2O_2 lesions. Because cells contain proteins for protection against oxidative agents and for repair of damage that these agents produce, we first examined whether a deletion mutant shows increased DNA repair capacity. It has been demonstrated that cells with increased repair capacity are able to repair H_2O_2-treated λ phage more efficiently, resulting in increased λ survival (8). Following growth to stationary phase in LB medium, cells were

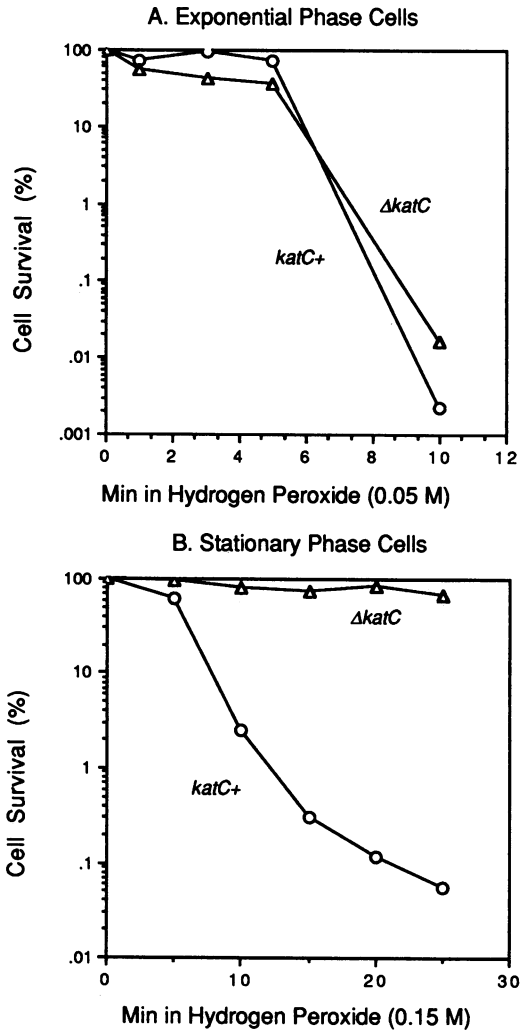

FIG. 2. Comparison of H_2O_2 sensitivity of cells grown to mid-exponential phase (A) and cells grown to stationary phase (B). Strains studied include MV1161 (parent) (○) and MV2640 [Δ(*argF-lacZ*)*205* (U169)] (△).

infected with H_2O_2-treated λ phage. There was no difference in the survival of H_2O_2-treated λ plated on either *katC*$^+$ or Δ*katC* (data not shown). From this result, we conclude that the H_2O_2 resistance phenotype is unlikely to result from enhanced repair of H_2O_2 lesions but may result from either an additional H_2O_2 protective mechanism, a more efficient function of a mechanism active in wild-type cells, or the loss of an H_2O_2 sensitization mechanism.

Mutant characterization. There are two general categories of peroxide resistance genes known: those that are functional in actively growing cells, and those that are active only during stationary phase. The stationary-phase-specific H_2O_2 protective genes require *katF* for their expression. *katF* encodes an alternative σ factor, σ^S, which is required to transcribe 30 to 50 genes expressed only in stationary phase (1, 2, 4, 14, 21, 22, 27). Because we initially tested only stationary-phase cells for H_2O_2 resistance, we investigated exponential-phase cells as well to determine whether the H_2O_2 resistance was unique to stationary phase. The results presented in Fig. 2 show that in exponential phase, mutant and parent strains were equally

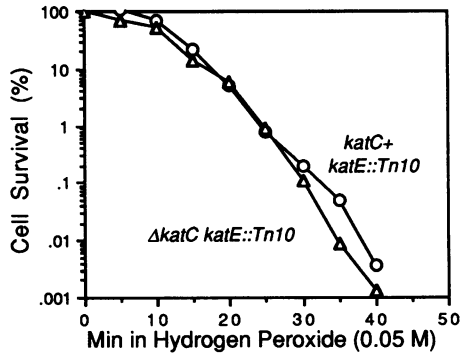

FIG. 3. Effects of *katE* on H_2O_2 sensitivity of parent and Δ(*argF-lacZ*)*205*(U169) strains grown to stationary phase. The strains studied include MV2922 (*katE12*::Tn*10*) (○) and MV2923 [Δ(*argF-lacZ*)*205* (U169) *katE12*::Tn*10*] (△).

sensitive to H_2O_2. As they entered stationary phase, the resistance of both parent and mutant strains increased dramatically. Higher H_2O_2 concentrations and longer exposure times were required to attain the same level of lethality in stationary-phase cells compared with exponential-phase cells (compare Fig. 2A and B). However, the mutant carrying Δ*katC* was significantly more resistant than the parent, indicating that the deletion enhanced stationary-phase H_2O_2 resistance.

The high-resistance phenotype requires *katE* and *katF*. σ^S is required for the expression of many stationary-phase-specific genes; therefore, we tested the effect of a *katF*::Tn*10* mutation on the Δ*katC* mutant phenotype. Both mutant and parent strains were sensitized to the lethal effects of peroxide as a result of a *katF13*::Tn*10* mutation, and more importantly, the resistance of the mutant strain no longer exceeded that of the parent, since their survival levels after a 5-min treatment with 0.05 M H_2O_2 were 50% for the *katF* mutant and 8% for the *katF* Δ*katC* mutant. Because the deletion may affect the expression or activity of a *katF*-dependent gene, we tested *katE*, encoding the stationary-phase catalase HPII (21). A *katE*::Tn*10* mutation negated the enhanced resistance of the Δ*katC* strain, making the mutant and parent strains equally sensitive to lower concentrations of H_2O_2 (Fig. 3). Thus, the enhanced resistance phenotype of the Δ*katC* strain requires functional *katE* and *katF* genes. Despite the requirement for both genes, catalase levels and the apparent K_m of the enzyme for H_2O_2 were the same in the parent and deletion mutants. For example, stationary-phase cultures of MV1161 and MV2640 contained 62.6 ± 3 and 60.5 ± 3 units of catalase per mg (dry weight) of cells, respectively. Furthermore, expression of *katE*, as examined by using a *katE*::*lacZ* fusion, was unaltered by the presence of F′ *lac*$^+$ *pro*$^+$ *katC*$^+$ plasmid in the deletion mutant (data not shown).

***katC*::Tn*9* insertion mutations.** The Δ(*argF-lacZ*)*205*(U169) deletion mutation defines a region of approximately 1.5 min or 70 kb. To define the genetics of the stationary-phase resistance mutation Δ*katC* more precisely, we chose to produce insertion mutations because they often result in null mutations and are therefore likely to mimic the phenotype resulting from the deletion mutation. To produce the desired insertion mutants, we specifically targeted Tn*9* insertions to the *argF-lacZ* region, using the *katC*$^+$ strain, MV1161, to identify the H_2O_2-resistant insertion mutants. Two of the Tn*9* insertion mutants obtained by this procedure exhibited increased H_2O_2 resistance relative to MV1161 (Fig. 4) and mapped to the same locus. We chose one, *katC5*::Tn*9*, for further study.

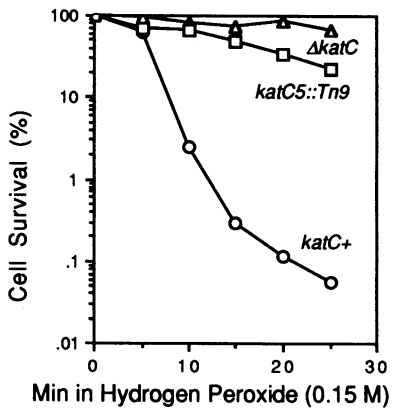

FIG. 4. Comparison of H_2O_2 sensitivity in the deletion and Tn*9* insertion mutants grown to stationary phase. The strains studied include MV1161 (parent) (○), MV2640 [Δ(*argF-lacZ*)*205*(U169)] (△), and MV2689 (*katC5*::Tn*9*) (□).

Cloning and DNA analysis of the *katC5*::Tn*9* mutation. The *katC5*::Tn*9* insertion mutation and flanking DNA were cloned from a *Bam*HI digest of chromosomal DNA, selecting for chloramphenicol resistance to yield pC3 (Fig. 5). A total of 4.2 kb of DNA surrounding Tn*9* was sequenced to identify the insertion site. The nonmutated region was also cloned on a *Bam*HI fragment by using DNA flanking the Tn*9* in pC3 as a probe to yield pC8 (Fig. 5). Relevant regions of pC8 were also sequenced for comparison with the Tn*9*-containing sequence (Fig. 6), revealing that Tn*9* had inserted into an existing IS*1* element, a frequent occurrence of Tn*9* transposition because of the IS*1* sequences at its ends (3). The DNA sequence also identified the specific insertion element as IS*1*B, which is fused to a 181-bp fragment of IS*30*B and which maps between *argF* and *proA* (30–32). The insertion of Tn*9* at this position and the resultant H_2O_2 resistance phenotype implicate IS*1*B or the IS*1*B-IS*30*B fusion as potentially playing a role in the H_2O_2 sensitization phenotype. A general picture of these clones and the site of Tn*9* insertion are shown in Fig. 5 and 6.

DISCUSSION

We have demonstrated that a gene, or genes, present in the *lacZ*-to-*proA* region of the *E. coli* K-12 chromosome confers a peroxide-sensitive phenotype. The deletion Δ(*argF-lacZ*)*205* (U169) (or Δ*katC*, since it deletes *katC*) and the Tn*9* insertion at IS*1*B both cause a peroxide-resistant phenotype in stationary-phase cells but not exponential-phase cells. A number of intriguing questions have been raised by this observation. One question is, why are catalase HPII levels unaffected by Δ*katC*, despite both *katF* and *katE* being involved in expression of the peroxide resistance phenotype? Several explanations for this contradiction are possible. For example, a masking of the additional resistance provided by the Δ*katC* mutation by either *katE* or *katF* mutants is a formal possibility but not too likely in light of the large difference in H_2O_2 sensitivity seen when the parent and deletion mutant are compared. Alternatively, catalase HPII may have a function in addition to its catalase activity, and this additional activity is affected by the *katC* gene.

A second question is, how does the Tn*9* impart the H_2O_2 resistance phenotype when it does not disrupt an open reading frame? The parental IS*1*B is retained in the *katC5*::Tn*9* mutant, and there is even a duplication of IS*1* because of the Tn*9* sequence. There is, however, one sequence change: the A at position 2024 of parent IS*1*B is changed to T in leftward IS*1*B. This produces a leucine-to-glutamine change in the putative transposase InsAB (10, 25, 38) produced by the leftward *insAB* gene (Fig. 6). However, the rightward IS*1*B retains the parental IS*1*B sequence. Therefore, if the A→T change contributes to the mutant phenotype, then the mutant product produced by the leftward IS*1*B must be dominant to that produced by the rightward IS*1*B. Several explanations are possible for this dominance. For example, the putative InsAB transposase may act as a multimer promoting sensitization, and the hybrid multimer composed of InsAB-L and InsAB-R protein (from the left and right IS*1* sequences, respectively, in Fig. 6) may be inactive. Alternatively, the chloramphenicol transacetylase (CAT) gene carried on Tn*9* may play a role interfering with expression of *insAB*. The CAT gene is transcribed from a strong promoter that directs transcription in the opposite direction of the *insAB* coding sequences. Transcription from the CAT promoter is known to read through the *insAB* sequence and repress cointegrate formation by IS*1* (18). It is therefore possible that CAT transcription produces an antisense RNA molecule, and such an antisense RNA could inhibit expression of *insAB* from both IS*1* elements. This argument also applies to open reading frame 1 (ORF-1) (Fig. 6), which may play a role in H_2O_2 sensitization.

A third question is, why do none of the six other IS*1* sequences on the *E. coli* chromosome affect peroxide sensitivity? Only one of the other six, IS*1*C, is identical to IS*1*B, and it is located on the opposite side of *argF* from IS*1*B such that it would also be deleted by the Δ(*argF-lacZ*)*205*(U169) deletion. IS*1*C should not be altered in the *katC5*::Tn*9* mutant strain in which IS*1*B is the site of insertion, but an antisense RNA, as

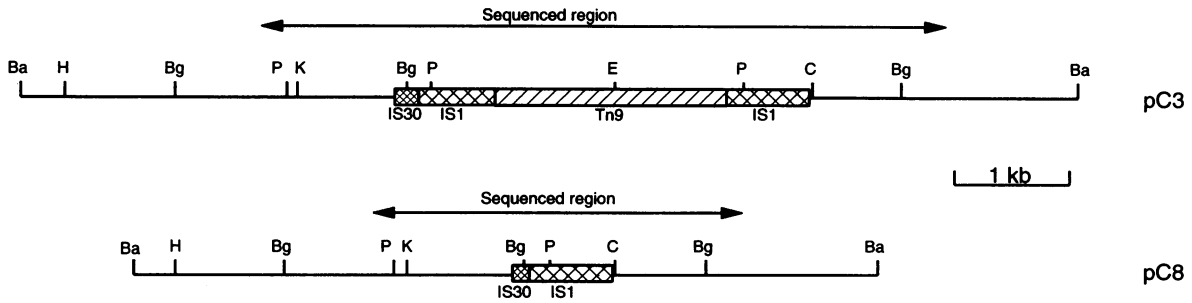

FIG. 5. Structures of plasmids used in this study. The vector plasmid was pBluescript SK+ (Stratagene). Abbreviations for restriction enzymes: Ba, *Bam*HI; H, *Hin*dIII; Bg, *Bgl*II; P, *Pst*I; K, *Kpn*I; E, *Eco*RI; C, *Cla*I. Locations of IS*1*B, IS*30*B, and Tn*9* sequences are indicated. Double-arrowed lines indicate the portions of the plasmids that were sequenced.

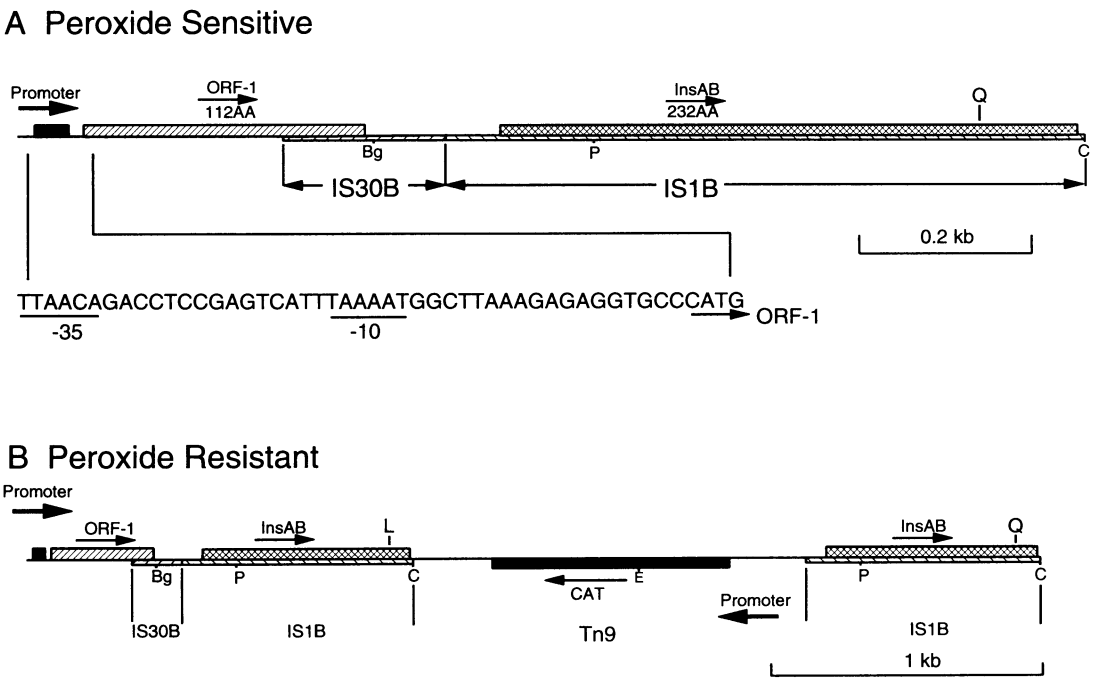

FIG. 6. Map of the *katC* region of the *E. coli* chromosome without (A) and with (B) Tn*9* present (the *katC5*::Tn*9* mutant allele). (A) Structure of the parent *katC* region which causes H_2O_2 sensitivity. The sequence of a potential promoter upstream of ORF-1 is indicated. Q, approximate location of a glutamine in the InsAB sequence. (B) Structure of the same area with Tn*9* inserted in the allele which causes peroxide resistance. Restriction enzyme designations are the same as in Fig. 5. Locations of the IS*1*B, IS*30*B, and Tn*9* sequences are indicated. Open reading frames corresponding to InsAB, CAT, and ORF-1 are indicated with arrows, and the promoters upstream of ORF-1 and the CAT gene are indicated with heavier arrows. The L above the leftward InsAB segment denotes a leucine at the same location where a glutamine (Q) is found in the rightward InsAB sequence and in the original InsAB sequence in panel A.

discussed above, would affect expression from IS*1*C as well. Therefore, we cannot formally exclude a role for IS*1*C in peroxide sensitivity. However, the remaining five IS*1* sequences are present in the Δ*katC* mutant but do not impart peroxide sensitivity. Either the sequence differences in these other five prevent the peroxide-sensitive phenotype from being expressed or a segment of DNA adjacent to IS*1*B, possibly ORF-1 formed in part by the fusion to IS*30*B, is required. Current experiments are directed at determining the potential role of ORF-1 and its promoter sequence which lie upstream of IS*1*B insert (Fig. 6) and at further analyzing the potential role of IS*1* itself in the H_2O_2 sensitization phenotype.

ACKNOWLEDGMENTS

This work was supported by NIH grant GM37052 to M.R.V. and NSERC grant OGP9600 to P.C.L. DNA sequence analysis was performed at the NCBI, using the BLAST network service.

REFERENCES

1. **Aldea, M., T. Garrido, C. Hernandes-Chico, M. Vicente, and S. R. Kushner.** 1989. Induction of a growth-phase-dependent promoter triggers transcription of *bolA*, an *Escherichia coli* morphogene. EMBO J. **8**:3923–3931.
2. **Atlung, T., A. Nielsen, and F. G. Hansen.** 1989. Isolation, characterization, and nucleotide sequence of *appY*, a regulatory gene for growth-phase-dependent gene expression in *Escherichia coli*. J. Bacteriol. **171**:1683–1691.
3. **Berg, C. M., and D. E. Berg.** 1987. Uses of transposable elements and maps of known insertions, p. 1071–1109. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
4. **Bohannon, D. E., N. Connell, J. Keener, A. Tormo, M. Espinosa-Urgel, M. M. Zambrano, and R. Kolter.** 1991. Stationary-phase inducible "gearbox" promoters: differential effects of *katF* mutations and role of σ^{70}. J. Bacteriol. **173**:4482–4492.
5. **Brot, N., L. Weissbach, J. Werth, and H. Weissbach.** 1981. Enzymatic reduction of protein-bound methionine sulfoxide. Proc. Natl. Acad. Sci. USA **78**:2155–2158.
6. **Crisona, N. J., J. A. Nowak, H. Nagaishi, and A. J. Clark.** 1980. Transposon-mediated conjugational transmission of nonconjugative plasmids. J. Bacteriol. **142**:701–713.
7. **Demple, B.** 1991. Regulation of bacterial oxidative stress genes. Annu. Rev. Genet. **25**:315–337.
8. **Demple, B., and J. Halbrook.** 1983. Inducible repair of oxidative DNA damage in *Escherichia coli*. Nature (London) **304**:466–468.
9. **Demple, B., and S. Linn.** 1982. 5,6-Saturated thymine lesions in DNA: production by ultraviolet light of hydrogen peroxide. Nucleic Acids Res. **10**:3781–3789.
10. **Escoubas, J. M., M. F. Prere, O. Fayet, I. Salvignol, D. Galas, D. Zerbib, and M. Chandler.** 1991. Translational control of transposition activity of the bacterial insertion sequence IS1. EMBO J. **10**:705–712.
11. **Farr, S. B., and T. Kogoma.** 1991. Oxidative stress responses in *Escherichia coli* and *Salmonella typhimurium*. Microbiol. Rev. **55**:561–585.
12. **Greenberg, J. T., and B. Demple.** 1989. A global response induced in *Escherichia coli* by redox cycling agents overlaps with that induced by peroxide stress. J. Bacteriol. **171**:3933–3939.
13. **Kaasen, I., P. Falkenberg, O. B. Tyrvold, and A. R. Strom.** 1992. Molecular cloning and physical mapping of the *otsBA* genes, which encode the osmoregulatory trehalose pathway of *Escherichia coli*: evidence that transcription is activated by KatF (AppR). J. Bacteriol. **174**:889–898.
14. **Lange, R., and R. Hengge-Aronis.** 1991. Identification of a central regulator of stationary-phase gene expression in *Escherichia coli*.

Mol. Microbiol. **5**:49–59.
15. **Loewen, P. C., and J. Switala.** 1987. Multiple catalases in *Bacillus subtilis*. J. Bacteriol. **169**:3601–3607.
16. **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.
17. **Low, K. B.** 1987. Hfr strains of *Escherichia coli* K-12, p. 1134–1137. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
18. **Machida, C., Y. Machida, H.-C. Wang, K. Ishizaki, and E. Ohtsubo.** 1983. Repression of cointegration ability of insertion element IS1 by transcriptional readthrough from flanking regions. Cell **34**:135–142.
19. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
20. **Mead, J. F.** 1976. Free radical mechanisms of lipid damage and consequences for cellular membranes, p. 51–68. *In* W. A. Pryor (ed.), Free radicals in biology. Academic Press, New York.
21. **Mulvey, M., J. Switala, A. Borys, and P. C. Loewen.** 1990. Regulation of transcription of *katE* and *katF* in *Escherichia coli*. J. Bacteriol. **172**:6713–6720.
22. **Mulvey, M. R., and P. C. Loewen.** 1989. Nucleotide sequence of *katF* of *Escherichia coli* suggests KatF protein is a novel σ factor. Nucleic Acids Res. **17**:9979–9991.
23. **Olsen, A., A. Arnqvist, M. Hammar, S. Sukupolvi, and S. Normark.** 1993. The RpoS sigma factor relieves H-NS mediated transcriptional repression of *csgA*, the subunit gene of fibronectin-binding curli in *Escherichia coli*. Mol. Microbiol. **7**:523–536.
24. **Sak, B. D., A. Eisenstark, and D. Touati.** 1989. Exonuclease III and the catalase hydroperoxidase II in *Escherichia coli* are both regulated by the *katF* gene product. Proc. Natl. Acad. Sci. USA **86**:3271–3275.
25. **Sekine, Y., and E. Ohtsubo.** 1992. DNA sequences required for translational frameshifting in production of the transposase encoded by IS1. Mol. Gen. Genet. **235**:325–332.
26. **Shellhorn, H. E., and V. L. Stones.** 1992. Regulation of *katF* and *katE* in *Escherichia coli* K-12 by weak acids. J. Bacteriol. **174**:4769–4776.
27. **Tanaka, K., Y. Takayanagi, N. Fujita, A. Ishihama, and H. Takahashi.** 1993. Heterogeneity of the principal σ factor in *Escherichia coli*: the *rpoS* gene product σ^{38}, is a second principal σ factor of RNA polymerase in stationary-phase *Escherichia coli*. Proc. Natl. Acad. Sci. USA **90**:3511–3515.
28. **Tormo, A., M. Almiron, and R. Kolter.** 1990. *surA*, an *Escherichia coli* gene essential for survival in stationary phase. J. Bacteriol. **172**:4339–4347.
29. **Touati, E., E. Dassa, J. Dassa, P. L. Boquet, and D. Touati.** 1991. Are *appR* and *katF* the same *Escherichia coli* gene encoding a new sigma transcription initiation factor? Res. Microbiol. **142**:29–36.
30. **Umeda, M., and E. Ohtsubo.** 1989. Mapping of insertion elements IS1, IS2 and IS3 on the *Escherichia coli* K12 chromosome: role of the insertion elements in formation of Hfrs and F′ factors and in rearrangement of bacterial chromosomes. J. Mol. Biol. **208**:601–614.
31. **Umeda, M., and E. Ohtsubo.** 1990. Mapping of insertion element IS30 in the *Escherichia coli* K12 chromosome. Mol. Gen. Genet. **222**:317–322.
32. **Umeda, M., and E. Ohtsubo.** 1991. Four types of IS1 with differences in nucleotide sequence reside in the *Escherichia coli* K-12 chromosome. Gene **98**:1–5.
33. **Volkert, M. R., and L. I. Hajec.** 1991. Molecular analysis of the *aidD6*::MudI(*bla lac*) fusion mutation of *Escherichia coli*. Mol. Gen. Genet. **229**:319–323.
34. **Volkert, M. R., L. I. Hajec, and D. C. Nguyen.** 1989. Induction of the alkylation-inducible *aidB* gene of *Escherichia coli* by anaerobiosis. J. Bacteriol. **171**:1196–1198.
35. **Volkert, M. R., and D. C. Nguyen.** 1984. Induction of specific *Escherichia coli* genes by sublethal treatments with alkylating agents. Proc. Natl. Acad. Sci. USA **81**:4110–4114.
36. **von Ossowski, I., M. R. Mulvey, P. A. Leco, A. Borys, and P. C. Loewen.** 1991. Nucleotide sequence of *Escherichia coli katE*, which encodes catalase HPII. J. Bacteriol. **173**:514–520.
37. **Walkup, L. K. B., and T. Kogoma.** 1989. *Escherichia coli* proteins inducible by oxidative stress mediated by the superoxide radical. J. Bacteriol. **171**:1476–1484.
38. **Zerbib, D., P. Poland, J. M. Escoubas, D. Galas, and M. Chandler.** 1990. The regulatory role of the IS1-encoded InsA protein in transposition. Mol. Microbiol. **4**:471–477.