JOURNAL OF BACTERIOLOGY, Oct. 1986, p. 13–21
0021-9193/86/100013-09$02.00/0

Vol. 168, No. 1

Control of Sensitivity to Inactivation by H_2O_2 and Broad-Spectrum Near-UV Radiation by the *Escherichia coli katF* Locus

LAURI J. SAMMARTANO, R. W. TUVESON,* AND R. DAVENPORT

Department of Genetics and Development, University of Illinois, Urbana, Illinois 61801

Received 18 February 1986/Accepted 30 June 1986

Mutations in the *Escherichia coli katF* gene (hydroperoxidase II) result in sensitivity to inactivation by H_2O_2 and broad-spectrum near-UV (NUV; 300 to 400 nm) radiation. Another mutation, *nur*, originally described as conferring sensitivity to inactivation by broad-spectrum and monochromatic NUV, also confers sensitivity to inactivation by H_2O_2. Genetic analysis via transduction suggests that the *nur* mutation is a mutant allele of the *katF* locus. As previously reported for broad-spectrum and monochromatic NUV wavelengths, the sensitivity of a particular strain to H_2O_2 inactivation is also independent of the *recA* and *uvrA* alleles. Extracts of *nur* and *katF* strains lack catalase (hydroperoxidase II) as revealed by polyacrylamide gels stained for such activity, which is consistent with the genetic results.

The mutagenic and inactivating effects of both monochromatic and broad-spectrum near-UV (NUV) wavelengths (300 to 400 nm) have been the subject of numerous investigations, which have been extensively reviewed (10, 13, 17, 18, 40).

A mutation in an *Escherichia coli* gene (*nur*) has been described which sensitizes cells to inactivation by NUV without affecting sensitivity to far-UV (FUV) inactivation (35–37). Specifically, it was shown that the *recA13*, *recA1*, and *uvrA6* mutations did not affect the sensitivity of stationary-phase cells to NUV inactivation. However, the *polA1* mutation did influence the sensitivity of *E. coli* cells to inactivation by NUV in an *nur*$^+$ genetic background (36). The fact that the *polA1* mutation sensitizes *E. coli* to NUV inactivation and that *E. coli xthA* mutants (exonuclease III deficient) are sensitive to inactivation by H_2O_2 (8) and NUV (31) might mean that repair of or protection against NUV- and H_2O_2-induced damage is based on a complex oxidative defense system (4).

Further evidence that H_2O_2 is involved in NUV inactivation comes from the observation that incorporation of bovine catalase into the plating medium or the irradiated cell suspension protects *E. coli* cells from both inactivation and mutagenesis by broad-spectrum NUV (32). Hartman (14) has also presented evidence that H_2O_2 is involved with NUV inactivating events in stationary-phase *E. coli* cells.

Pretreatment of *E. coli* or *Salmonella typhimurium* cells with a sublethal concentration of H_2O_2 results in protection against inactivation by a lethal concentration of H_2O_2 (4, 7) as well as by broad-spectrum NUV (33, 39). Tyrrell (39) has shown that pretreatment of growing *E. coli* cells with low fluences of NUV protects against inactivation by H_2O_2. Christman et al. (4) have demonstrated that 30 proteins, including catalase and superoxide dismutase, are induced during adaptation to H_2O_2 in *S. typhimurium*.

If H_2O_2 were one product of NUV irradiation in cells, it would be expected that cells lacking catalase should be sensitive to inactivation by NUV. Recently, Leowen and his colleagues have described mutants which are defective in catalase activity (21–23). In this paper, we present evidence that lesions in the *katF* gene, but not the *katE* or *katG* gene, result in sensitivity to broad-spectrum NUV as well as to H_2O_2 independent of the *recA1* and *uvrA6* mutations. Genetic experiments are described which indicate that the *nur* mutation (35–37) is an allele of the *katF* gene. Strains carrying mutations in the *katF* gene lack the catalase activity designated hydroperoxidase II (HP-II) (5), as demonstrated by polyacrylamide gel electrophoresis.

* Corresponding author.

MATERIALS AND METHODS

Strains. The bacterial and bacteriophage strains used in this investigation are described in Table 1.

Chemicals. The reagent 30% H_2O_2 used was obtained from E. K. Industries, Inc. (10 M concentration). The glucose oxidase used in the H_2O_2-generating system was type X from *Aspergillus niger*, obtained from Sigma Chemical Co., St. Louis, Mo.

Media. The complex medium was Luria-Bertani (LB) broth, containing (per liter) 10 g of tryptone (Difco Laboratories), 5 g of yeast extract (Difco), and 10 g of NaCl, solidified as required with 12 g of agar (Difco) (27). The minimal medium used to assess response to broad-spectrum NUV and H_2O_2 inactivation was appropriately supplemented minimal A medium (27) solidified with 1.2% agar (Difco).

Transduction. The transducing phage (P1 Cm *clr-100*) is the same one used in previous experiments (20, 36, 37). The methods for producing lysogens, transducing lysates, and selecting transductants have been described previously (27, 29, 30).

The method used to transduce the *polA12* mutation from strain MM383 was identical to that used previously to transfer the *polA1* mutation (6) from *E. coli* p3478 to appropriate recipients (36). Briefly, strain RT12 was transduced to *metE*$^+$ by using transducing bacteriophage (P1 Cm *clr-100*) prepared on strain MM383 (*polA12*). Of 345 *metE*$^+$ transductants, 4 represented cotransductants for *polA12* as judged by their sensitivity to FUV inactivation (cotransduction frequency, 1.2%; *metE*$^+$ transductants per viable cell, 2.0×10^{-7}; transductants per viable bacteriophage particle, 6.9×10^{-7}). One of the *polA*$^+$ *metE*$^+$ transductants was selected at random for use in all subsequent experiments and designated RT16(1). This strain is genetically identical to strain RT16 (36), but since it was derived from an independent transduction experiment, it was given the designation (1) to indicate its independent origin. As far as NUV and FUV sensitivity,

TABLE 1. Bacterial strains and bacteriophage

Strain or phage	Relevant genotype	Reference or source
E. coli strains		
AT713	F⁻ *thi-1 argA21 cysC43 lysA22 mtl-2 xyl-7 malA1 str-104 nur*⁺ λ^r λ⁻ *supE44*	37
AB1157	F⁻ *thr-1 leu-6 thi-1 argE3 his-4 proA2 lacY1 galK2 mtl-1 xyl-5 ara-14 rpsL31 tsx-33* λ⁻ *supE44*	9
RT2	F⁻ *thi*⁺ *argA21 lysA22 mtl-2 xyl-7 malA1 str-104* λ^r λ⁻ *supE44? nur*	37
RT8	F⁻ *thi*⁺ *argA21 lysA22 mtl-2 malA1 str-104* λ^r λ⁻ *supE44? nur*	35
RT10	Same as RT8 except *nur*⁺	35
RT8 *katE*	Same as RT8 except *katE12*::Tn*10*	This study
RT8 *katF*	Same as RT8 except *katF13*::Tn*10*	This study
RT8 *katG*	Same as RT8 except *katG17*::Tn*10*	This study
RT10 *katE*	Same as RT10 except *katE12*::Tn*10*	This study
RT10 *katF*	Same as RT10 except *katF13*::Tn*10*	This study
RT7h (Tet^r)	F⁻ *thi-1 argA21 lysA22 mtl-2 malA1 str-104* λ^r λ⁻ *supE44?*	20
RT8 (Tet^r)	Same as RT7 (Tet^r) except *thi*⁺ *uvrA*⁺	20
RT9 (Tet^r)	Same as RT7 (Tet^r) except *nur*⁺	20
RT10 (Tet^r)	Same as RT7 (Tet^r) except *thi*⁺ *uvrA*⁺ *nur*⁺	20
MM383	F⁻ *lacZ53 rpsL151 thyA36 rha-5 deoC2* λ⁻ Δ(*rrnD-rrnE*) *polA12*	28
RT12	F⁻ *thi-1 argA21 lysA22 metE46 xyl-7 malA1 str-104* λ^r λ⁻ *supE44? nur*⁺	36
RT15	Same as RT12 except *metE*⁺ *polA1*	36
RT16(1)	Same as RT12 except *metE*⁺ *polA*⁺	36; this study
RT17	Same as RT12 except *metE*⁺ *polA12*	This study
KL16-99	Hfr PO61; *thi-1 relA1 recA1* λ⁻ *nur*	24
UM120	Hfr H; *thi-1 katE12*::Tn*10*	22
UM122	Hfr H; *thi-1 katF13*::Tn*10*	22
UN202	Hfr H; *thi-1 katG17*::Tn*10*	23
Bacteriophage		
P1	Tn*9* Cm^r *clr-100*	29, 30

as well as all other markers, are concerned, this transductant proved indistinguishable from the original RT16 transductant and was used in the experiments described in this report. Among the four *metE*⁺ transductants which were identified as carrying *polA12* based on their sensitivity to FUV, one was selected for use in subsequent experiments and designated RT17.

H_2O_2 dose-response curves. Cells were grown at 37°C with shaking in sidearm flasks (Belco) containing 50 ml of LB broth to stationary phase as described previously (37, 38). A 5-ml portion of cells was washed three times in cold saline, and 3 ml of the washed cells were then added to 10 ml of cold saline. Nine milliliters of this suspension was withdrawn and placed in a sterile 125-ml flask. To initiate the experiment, 1.0 ml of a 500 mM solution of H_2O_2 was added to the flask. The flask was shaken, and a 0.1-ml sample was immediately taken to estimate initial survival. The cell suspension prepared in this manner contained between 3×10^8 and 5×10^8 cells per ml. At specified time intervals, 0.1-ml samples were removed, diluted appropriately, and plated on minimal medium to assess viability.

H_2O_2-generating system. The H_2O_2-generating system used was the glucose-glucose oxidase system modified from the one described by Klebanoff (19). *E. coli* cells were grown to stationary phase and washed in saline as outlined above. However, for the last wash the cells were brought up in 100 mM sterile glucose dissolved in 0.85% saline, and 9.0 ml was transferred to a sterile flask. Glucose oxidase (Sigma) was made in saline at approximately 10,000 U/ml (1 U of glucose oxidase catalyzes the formation of 1 μmol of gluconic acid and H_2O_2 per min from glucose). One milliliter of the glucose oxidase was added to the 9.0 ml of cell suspension, and a 0.1-ml portion was immediately taken and plated to assess initial survival. The reaction mixture prepared in this way is expected to generate 1 mmol of H_2O_2 per min. The reaction flask was shaken at 37°C, and portions were removed at appropriate intervals and plated to assess survival. All plates were incubated for 48 h at 37°C.

Broad-spectrum NUV fluence response curves. Stationary-phase cells treated with broad-spectrum NUV were prepared as described previously (32). The broad-spectrum NUV source used was identical to that used in all our previous experiments involving broad-spectrum NUV (37). The source consists of a bank of four lamps (GE 40BLB, integral filter) with a range of emission from 300 to 400 nm, with a maximum emission at 350 nm. These lamps emit 97% of their radiant energy between 300 and 400 nm. However, we have measured the fluence rate with a DRC-100X digital radiometer equipped with a DIX-365 sensor (Spectroline) and found it to be 24 rather than 10 W/m^2 as previously reported (32).

Preparation of cellular extracts for polyacrylamide catalase activity gels. Strains to be tested for catalase activity were grown overnight in 200 ml of LB broth at 30°C. The stationary-phase cells were harvested by centrifugation at 10,000 × *g* for 10 min, suspended in 0.0625 M Tris hydrochloride buffer, pH 6.8, containing 10^{-4} M EDTA, and washed twice in the same buffer. The bacterial cells were ruptured by subjecting the suspensions twice to 15,000 lb/in^2 in a French pressure cell (American Instrument Co., Urbana, Ill.) at a flow rate of 1 ml/min. Cell debris was removed by centrifugation at 12,000 × *g*. The cell extracts were stored on ice. Protein content was estimated by standard procedures (25).

Visualization of catalase and peroxidase on polyacrylamide gels. For visualization of catalase and peroxidase, 50 μg of crude extract was run on 7.5% polyacrylamide gels with the nondissociating, discontinuous buffer described previously (11), except that the resolving buffer was pH 8.0. The activity stain used to localize catalase was the one described by Harris and Hopkinson (12). Briefly, the gels were rinsed in distilled water after electrophoresis and soaked in a 0.1%

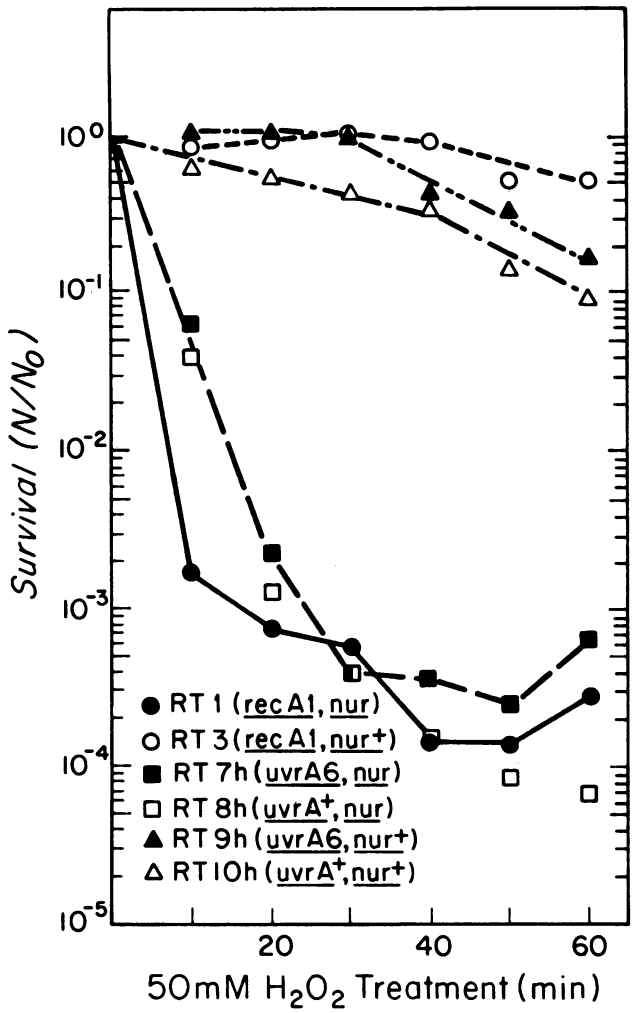

FIG. 1. Survival responses of various *E. coli* strains when treated with 50 mM H_2O_2.

H_2O_2 solution for 15 min, rinsed twice with distilled water, and soaked in a 50:50 mixture of freshly prepared 2% $FeCl_3$ and $K_6(FeCN)_6$ for 30 s with gentle agitation.

RESULTS

H_2O_2 inactivation of DNA repair-deficient strains. Since it has been shown that *E. coli xthA* mutants (exonuclease III deficient) are sensitive to H_2O_2 (8) and to broad-spectrum NUV (31), it was considered important to test whether another mutation (*nur*) which sensitizes cells to NUV (35–37, 41) might sensitize cells to H_2O_2 inactivation. The *nur* mutation does not sensitize *E. coli* cells to FUV inactivation even in combination with genes which do confer sensitivity to FUV (*recA1* and *recA13* [37] and *uvrA6* [20, 37]). To test the possibility that genes which confer sensitivity to FUV might confer sensitivity to H_2O_2 inactivation, strains differing in FUV sensitivity (*recA1* and *uvrA6*) were included among those tested. Figure 1 presents the 50 mM H_2O_2 dose-response curves obtained with strains which have been used previously in experiments with NUV. It should be noted that even though both strains RT1 and RT3 carry the *recA1* mutation conferring sensitivity to FUV, RT3 was resistant to 50 mM H_2O_2 inactivation. Therefore, the *nur* allele must be the factor which determines the sensitivity of these strains to H_2O_2 inactivation. Similarly, strains RT7h and RT8h were sensitive to H_2O_2 inactivation, while strains RT9h and RT10h were resistant to inactivation by 50 mM H_2O_2, reflecting the fact that they carry the *nur*$^+$ allele. For these strains, the fact that RT7h and RT9h carry the *uvrA6* mutation did not influence their response to 50 mM H_2O_2 treatment. For those strains carrying the *nur* allele, RT1, RT7h, and RT8h, the kinetics of H_2O_2 inactivation were initially exponential (or apparently so), followed by a "tail" in the inactivation curve. These kinetics may be explained by assuming that the initial 50 mM H_2O_2 concentration is reduced over time by the enzymatic activity of the cells in suspension (i.e., catalase and peroxidase). If this were true, it would be expected that the kinetics of inactivation would be strictly exponential for a strain carrying the *nur* mutation if H_2O_2 were continually generated within the suspension. Therefore, strains RT7h (*nur*) and RT9h (*nur*$^+$) were suspended in an enzyme reaction mixture designed to generate 1 mM of H_2O_2 per min as a product of the glucose oxidase reaction (glucose + O_2 → gluconic acid + H_2O_2 [19]). The results of these experiments are presented in Fig. 2. It is apparent that the kinetics of inactivation for strain RT7h (*nur*) were exponential after the first 10 min of treatment under these conditions. The *nur*$^+$ strain (RT9h) was clearly resistant to H_2O_2 inactivation, whether H_2O_2 was generated continuously (Fig. 2) or held to a single initial concentration (50 mM; Fig. 1).

It has been demonstrated that the *polA1* mutation sensitizes *E. coli* cells to inactivation by NUV, but only in an *nur*$^+$ background (36). This was interpreted to mean that the *nur* gene is epistatic to *polA1*. Therefore, to test whether *polA* mutations sensitize cells to inactivation by 50 mM H_2O_2, the experiments had to be done in a *nur*$^+$ background.

Two *polA* alleles were tested for their sensitivity to 50 mM H_2O_2 inactivation. Strain RT17 (*polA12*) carried a temperature-sensitive allele of the *polA* locus and proved to be sensitive to both FUV and NUV inactivation as expected (28; data not shown). We confirmed that *polA12* cells are partially Pol$^-$ (28), since survival was only marginally enhanced when FUV- or NUV-treated cells were plated and incubated at 30°C rather than at 37°C. Both *polA* mutant alleles led to sensitivity to 50 mM H_2O_2 inactivation (Fig. 3). Again, the survival curves for the *polA* mutants exhibited an initial decline, followed by a tail. In fact, RT17 exhibited some apparent recovery between the 10- and 40-min sampling points. If the tailing in the survival curve represents depletion of the H_2O_2, as suggested above (Fig. 2), then readdition of H_2O_2 to a cell suspension should result in a further exponential decline in survival. This proved to be the case when a second portion of 500 mM H_2O_2 (diluted to 50 mM) was added to the RT15 cell suspension after an initial 60 min of incubation. This experiment, and the results involving the glucose-glucose oxidase H_2O_2-generating system (Fig. 2), supports the hypothesis that H_2O_2 is being removed from the cell suspension by activities of the cells themselves.

NUV and H_2O_2 sensitivity of catalase mutants. Loewen and colleagues have described mutants which affect catalase (HP-II) and peroxidase (HP-I) activities in *E. coli* (21–23). If NUV irradiation of *E. coli* cells generates H_2O_2, it would be expected that mutants lacking catalase activity should be sensitive to inactivation by NUV wavelengths. With the three strains developed by Loewen and his colleagues, which carried insertion mutations (Tn*10*) in each of the three genes controlling catalase (HP-II; strains UM120 [*katE12*] and UM122 [*katF13*]) and peroxidase activity (HP-I; strain

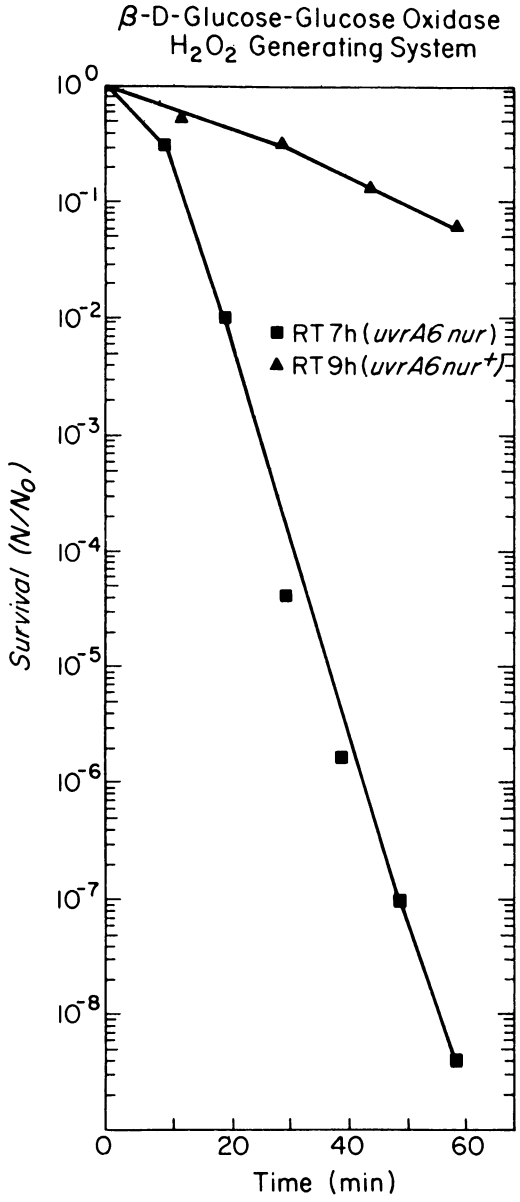

FIG. 2. Survival of an *nur* and an *nur*$^+$ strain in a beta-D-glucose oxidase H_2O_2-generating system designed to generate 1 mmol of H_2O_2 per min.

UM202 [*katG17*]), transducing particles (P1 Cm *clr-100*) were prepared and used to transduce strains RT8 and RT10 to *katE katF katG* by selecting for tetracycline resistance (Tn*10*). The two parent strains and each of the six transductants were treated with NUV and H_2O_2 (glucose-glucose oxidase H_2O_2-generating system). The results of the broad-spectrum NUV and H_2O_2 inactivation experiments with RT10 and the three Tn*10* (*kat*) transductants are presented in Fig. 4 and 5, respectively. In an *nur*$^+$ background, the *katF* insertion mutation led to sensitivity to both broad-spectrum NUV and H_2O_2 inactivation. When RT8 (*nur*) and its three Tn*10* (*kat*) transductants were tested for sensitivity to broad-spectrum NUV and H_2O_2 inactivation, the strains appeared to be equally sensitive to inactivation by H_2O_2, while RT8 *katG* was slightly more sensitive to NUV inactivation (Fig. 6 and 7). With the exception of the RT8 *katG* strain, RT8 and its derivative transductants were about as sensitive to broad-spectrum NUV inactivation as RT10 *katF* was (compare Fig. 4 and 6).

Transductional analysis testing the hypothesis that *nur* represents an allele of the *katF* locus. The results of the broad-spectrum NUV and H_2O_2 inactivation experiments suggest that both *katF13* and *nur* may represent mutant alleles of the *katF* locus. Previous attempts to map the *nur* allele by transduction were unsuccessful, but mating experiments placed the allele in the 50- to 60-min area of the *E. coli* linkage map (37). Loewen and Triggs (22) reported that *cysH* was about 49% cotransducible with *katF13* (min 59.2). Therefore, we transduced strain AT713 to *cysC*$^+$ (another gene in the cysteine operon) with P1 Cm *clr-100* transducing bacteriophage prepared on strain UM122 (*katF*::Tn*10*) and tested for tetracycline resistance (Table 2). Tet^r proved to be about 30.6% cotransducible with *cysC*$^+$. If *nur* represents an allele of the *katF* gene, then *nur* should cotransduce with *cysC*$^+$ at about 30%. We prepared transducing particles on strains RT2 (*nur*) and RT7h (*nur*) and transduced AT713 to *cysC*$^+$ in two independent experiments (Table 2). Each *cysC*$^+$ transductant had to be tested for its sensitivity to

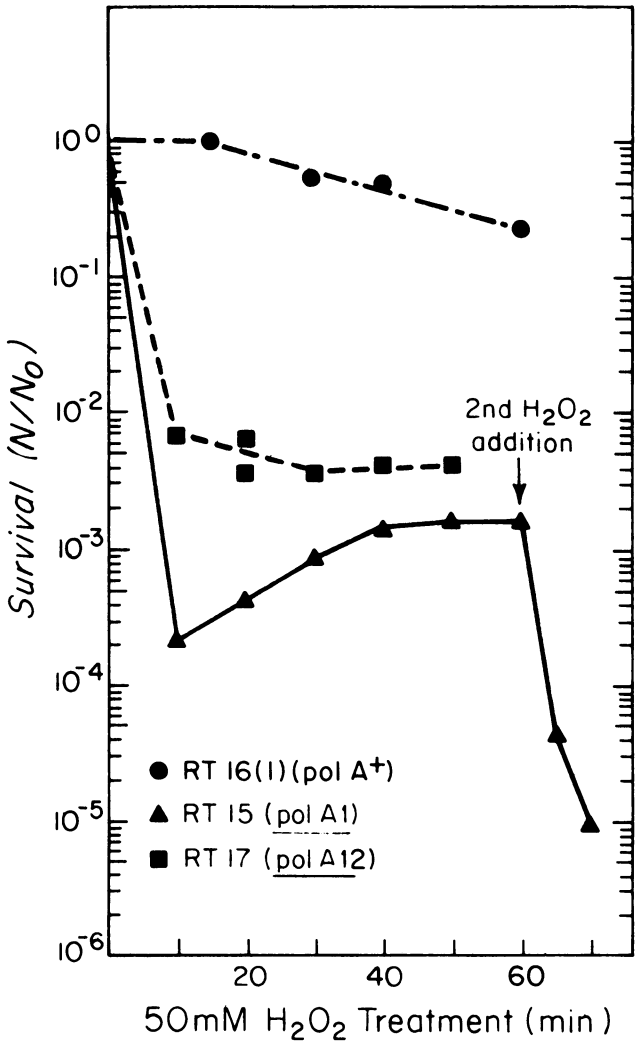

FIG. 3. Survival responses of three *E. coli* strains treated with 50 mM H_2O_2. In a single experiment involving strain RT15 (*polA1*), a second portion of H_2O_2 was added following a 60-min incubation.

TABLE 2. Results of transducing *E. coli* AT713 to *cysC*$^+$ with phage P1 Cm *clr-100* prepared on four different donors[a]

Expt	Donor strain	Total no. of *cysC*$^+$ transductants tested	Nonselected marker	No. (%) of transductants with nonselected marker	*P* vs UM122
1	UM122	147	Tet^r (*katF*)	45 (30.6)	
2	RT7h	21	*nur*	5 (23.8)	$0.7 > P > 0.5$
3	RT2	21	*nur*	4 (19.0)	$0.3 > P > 0.2$
4	KL16-99	21[b]	*nur*	0	>0.01

[a] Genetic maps of the markers carried by the strains used (numbers in parentheses for strain AT713 are the positions of the markers [in minutes] on the *E. coli* linkage map): AT713, *lysA22* (61.5)-*argA21* (60.5)—*katF* (58.9)-*recA*$^+$ (58.2); RT2, *lysA22-argA21-nur* (*katF*?)-*recA*$^+$; RT7h, *lysA22-argA21-nur* (*katF*?)-*recA*$^+$; KL16-99, *lysA*$^+$-*argA*$^+$-*nur* (*katF*?)-*recA1*. Suggested map for KL16-99, with break points for inversion indicated by X: *lysA*$^+$-*argA*$^+$-X-*recA1-nur* (*katF*?)-X.
[b] 247 tested previously (37).

NUV inactivation with a single fluence of NUV (864 kJ/m^2). This NUV fluence would be expected to produce about one cycle of inactivation in an *nur*$^+$ strain, while about four cycles of inactivation would be expected for an *nur* strain. Of 21 *cysC*$^+$ transductants tested in this fashion, 5 proved to be *nur* (23.8%) for transducing particles prepared on strain RT7h. Four of 21 (19.0%) proved to be *nur* for particles prepared on strain RT2 (Table 2). A 2 × 2 contingency table analysis was done to compare the proportion of Tet^r (*katF*) cotransductants in experiment 1 (Table 2) and the proportion of *nur* cotransductants in experiments 2 and 3 (Table 2). Based on this analysis, the proportion of cotransductants in experiments 2 and 3 did not differ significantly (at the 0.05 level) from the proportion of cotransductants observed in experiment 1 (Table 2). The *nur* mutation was originally isolated among *cysC*$^+$ recombinants from a mating between strains AT713 (F$^-$ and KL16-99 (Hfr PO61 [37]). When transducing particles (P1 Cm *clr-100*) were prepared on this strain and used to transduce AT713 to *cysC*$^+$, none of 247 transductants tested proved to be *nur*. We concluded from these tranduction and specific mating experiments that *nur* was located at about min 52 on the *E. coli* linkage map. It was entirely possible that the original transductants were incorrectly scored. Therefore, the transduction was repeated with KL16-99 as the donor. The resulting *cysC*$^+$ transductants were scored with a single fluence of NUV (864 kJ/m^2) for testing sensitivity to NUV inactivation (Table 2). Of 21 *cysC*$^+$ transductants, none proved to be NUV sensitive (*nur*, Table 2, experiment 4). This is consistent with previously reported results (37). To test whether this result was simply a matter of chance, a 2 × 2 contingency table analysis was done to compare the proportion of Tet^r(*katF13*; Table 2,

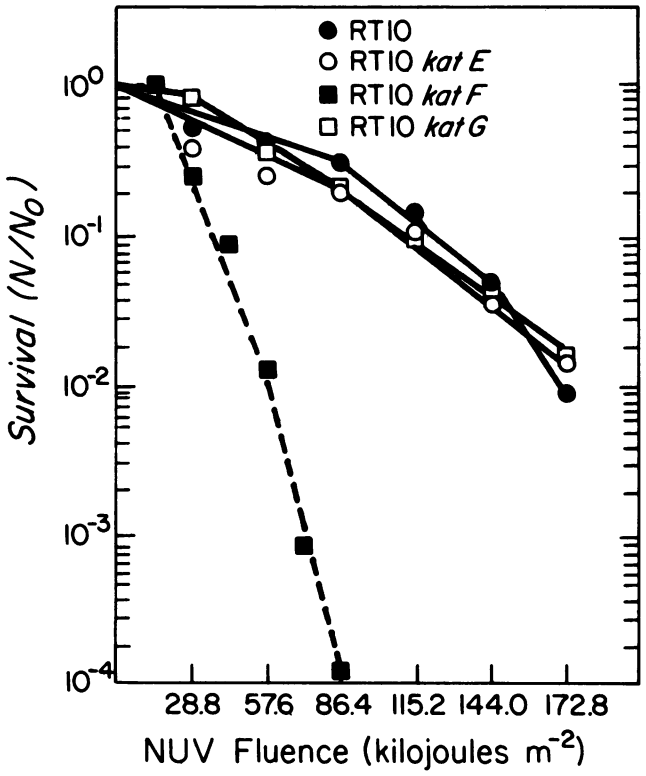

FIG. 4. Fluence survival responses of strain RT10 and three *kat* (insertion mutations) derivatives of this strain.

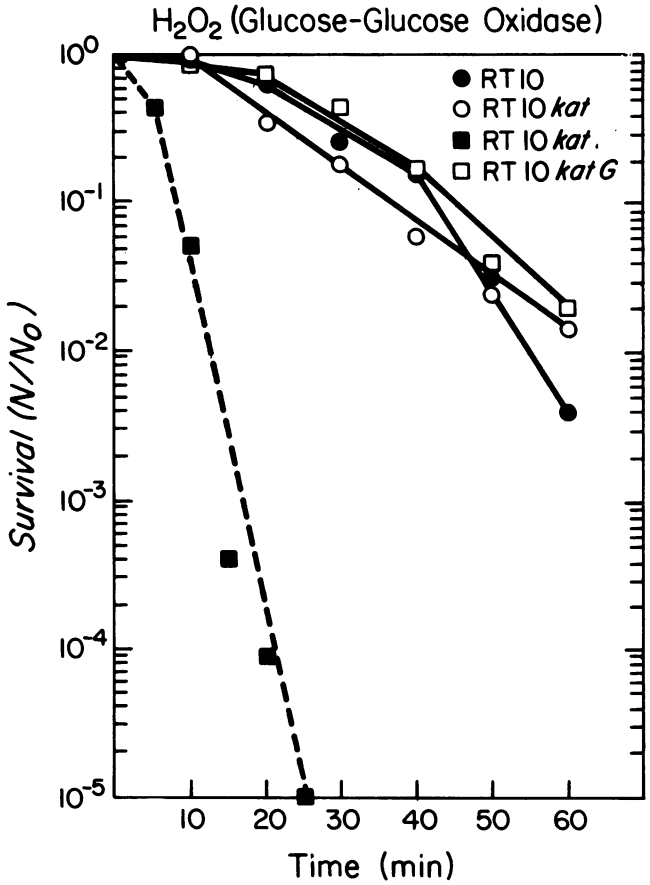

FIG. 5. Survival responses of strain RT10 and three *kat* (insertion mutations) derivatives of this strain when suspended in a beta-D-glucose oxidase-generating system designed to generate 1 mmol H_2O_2 per min.

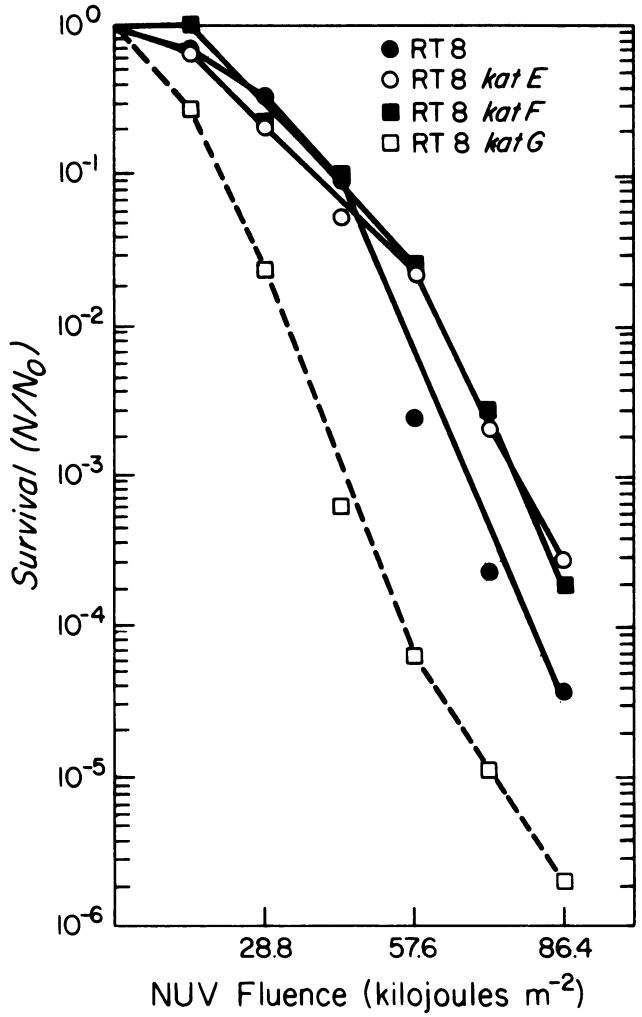

FIG. 6. Fluence survival responses of strain RT8 and three *kat* (insertion mutations) derivatives of this strain. Note the sensitivity of the *katG* strain.

experiment 1) cotransductants with bacteriophage prepared on strain UM122 with the proportion of *nur* cotransductants with bacteriophage prepared on strain KL16-99. Based on this analysis, the probability that the absence of *nur* cotransductants when selecting for $cysC^+$ with transducing particles prepared on strain KL16-99 was a matter of chance was less than 0.01. In Fig. 8, the results of a complete NUV inactivation experiment are presented for representative transductants from each of the four transduction experiments just described. In three of the experiments (1, 2, and 3), NUV-resistant and NUV-sensitive transductants were obtained (transducing particles prepared on strains RT2, RT7h, and UM122), while in the fourth experiment, only NUV-resistant strains were seen (transducing particles prepared on strain KL16-99).

Visualization of catalase and peroxidase enzyme activity on polyacrylamide gels. If *nur* is an allele of the *katF* gene, it would be expected that extracts from strains RT7 (*nur*) and RT8 (*nur*) would not exhibit the catalase (HP-II) band when stained for catalase activity on polyacrylamide gels (21–23). This expectation was confirmed (Fig. 9, lanes A and B). Note that the extracts from the nur^+ strains RT9 (lane C) and RT10 (lane D) exhibited both HP-I and HP-II activities. The pattern of bands seen in extracts of the RT8 (lane G) and RT10 (lane H) transductants with Tn*10* insertions in the *katF* gene was indistinguishable from the pattern seen with extracts of RT7 and RT8 (lanes A and B). When strain RT8 was transduced to *katG*, neither HP-I nor HP-II activity was found (lane I). On the other hand, RT10 transduced to *katG* did exhibit HP-II activity (lane J). Strain AB1157, which we have found to be NUV sensitive, exhibited little HP-II activity (lane K) (37). Finally, strain KL16-99 exhibited both HP-I and HP-II activity, although it was NUV sensitive and is the strain from which the *nur* gene was derived (37). Although in previous gels, RT10 *katE* extracts contained both HP-I and HP-II activities, in this particular experiment

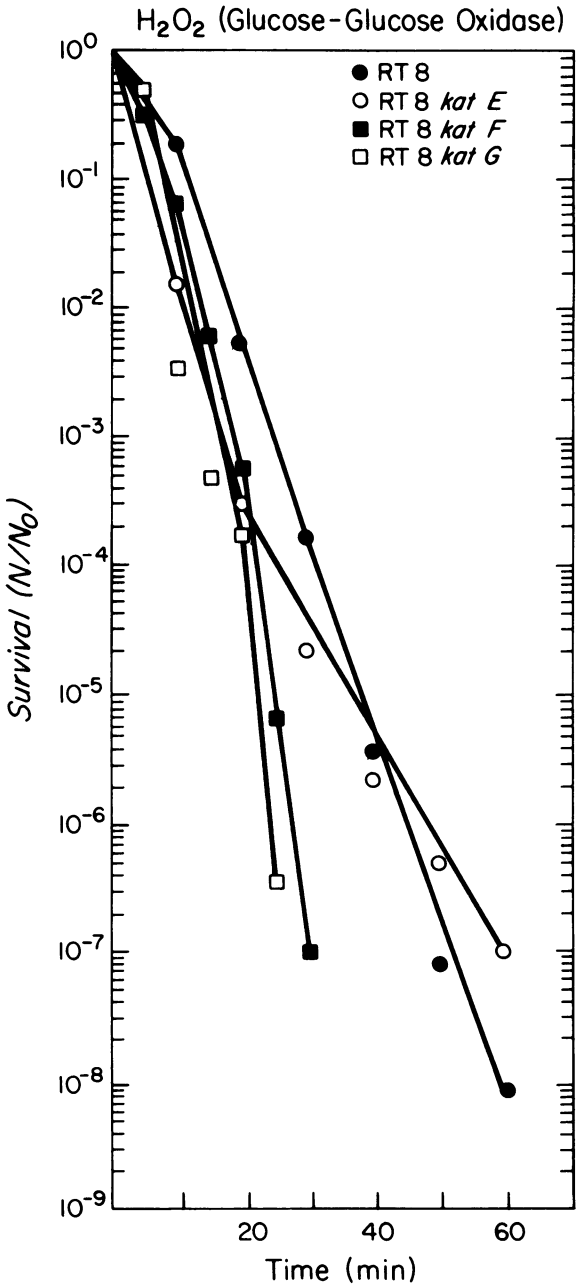

FIG. 7. Survival responses of strain RT8 and three *kat* (insertion mutations) derivatives when suspended in a beta-D-glucose oxidase-generating system designed to generate 1 mmol of H_2O_2 per min.

these activities were reduced (lane F). This probably reflects the fact that this extract had been frozen for some time before use in the experiment, while all other extracts had been freshly prepared.

Since the *katF* mutation sensitizes *E. coli* cells to inactivation by NUV and H_2O_2, it was considered possible that HP-I and HP-II activity might be present in different parts of the cell. Cell disruptions were done to determine whether these two activities might be differentially distributed in the cytoplasm and periplasmic space. Although extracts from the periplasmic fraction contained sufficient protein (50 μg/well), no detectable HP-I or HP-II activity was found associated with the periplasmic space (data not shown).

DISCUSSION

Suggestive evidence that H_2O_2 is involved in the mutagenic and inactivating effects of NUV has been present in the literature for some time (1, 26, 42, 43). A possible role for hydrogen peroxide in the mutagenic and inactivating effects of NUV was first suggested by the identification of the photoproduct resulting from the treatment of L-tryptophan with NUV as H_2O_2 (26, 43). It has been demonstrated that

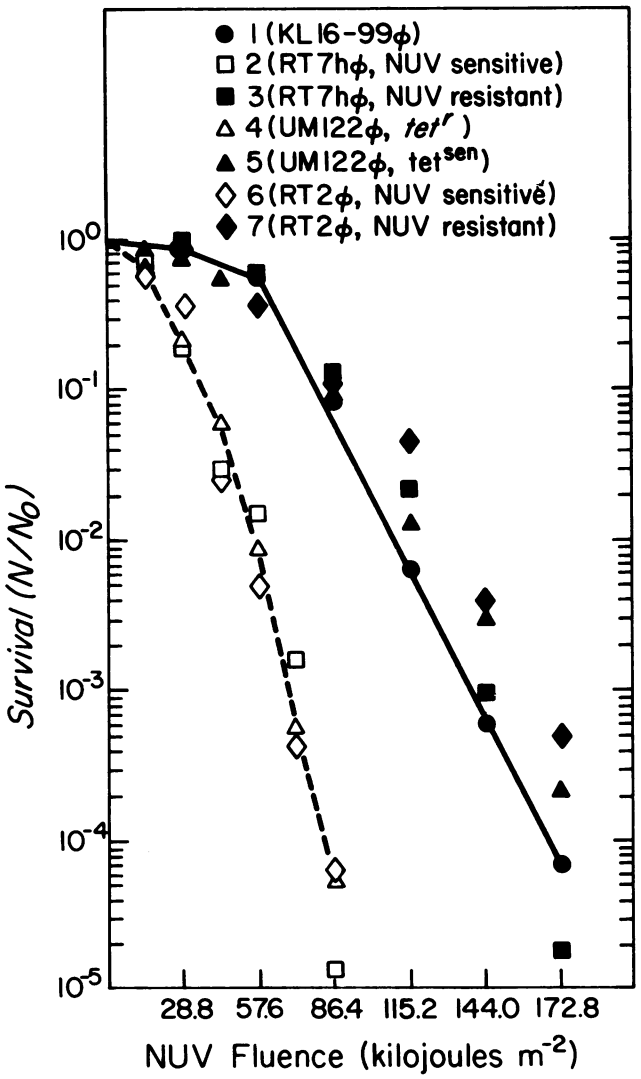

FIG. 8. Fluence survival responses of selected transductants from the four transduction experiments detailed in Table 2.

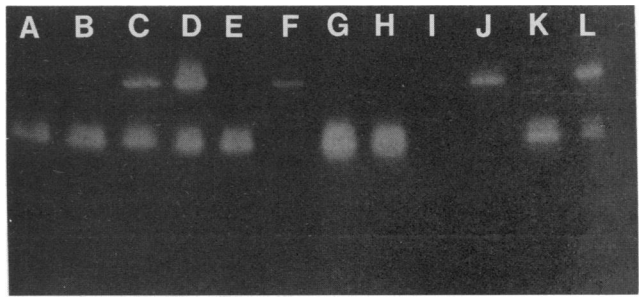

FIG. 9. Polyacrylamide gel stained for catalase (HP-II; upper band) and peroxidase (HP-I; lower band) enzymatic activity. Each well was loaded with 50 μg of crude extract. Extracts were obtained from (A) RT7 (*nur*), (B) RT8 (*nur*), (C) RT9 (*nur*$^+$), (D) RT10 (*nur*$^+$), (E) RT8 (*katE*), (F) RT10 (*katE*), (G) RT8 (*katF*), (H) RT10 (*katF*), (I) RT8 (*katG*), (J) RT10 (*katG*), (K) AB1157, and (L) KL16-99.

the tryptophan photoproduct (TP) (presumably H_2O_2) sensitizes DNA to the induction of single-strand DNA breaks by NUV, as well as sensitizing cells to NUV inactivation (42). Furthermore, Ananthaswamy and Eisenstark (2) have shown that concentrations of H_2O_2 which inactivate no more than 10 to 20% of bacteriophage particles sensitize both T-even and T-odd bacteriophage to inactivation by NUV as well as enhancing the yield of DNA strand breaks in bacteriophage T7. When *E. coli* cells were treated simultaneously with a sublethal concentration of H_2O_2 and NUV, Hartman and Eisenstark (15, 16) found synergistic killing, again implicating H_2O_2 in NUV effects.

Using DNA repair-deficient *E. coli* mutants, Ananthaswamy and Eisenstark (2) concluded that, for exponentially growing cells, the *polA*$^+$ and *recA*$^+$ functions are important in the repair of H_2O_2-induced single-strand DNA breaks. Based on work with 25 different *E. coli* strains carrying various combinations of DNA repair defects, Carlsson and Carpenter (3) concluded that the *recA*$^+$ gene product was more important than the level of catalase or superoxide dismutase in protecting exponentially growing cells against H_2O_2 inactivation.

If H_2O_2 plays a role in the inactivating and mutagenic effects of NUV and the *recA*$^+$ gene product is important in protecting against these agents, it might be anticipated that SOS functions would be induced by either H_2O_2 or NUV. Using a strain with the *lac* gene fused to the *recA* promoter, Turner and Eisenstark (34) have shown that selected NUV wavelengths and broad-spectrum solar UV light do not induce beta-galactosidase in the fusion strain. However, the enzyme was readily induced by FUV in the same fusion strain. In addition, lambda prophage induction and Weigle reactivation were not demonstrable with selected NUV wavelengths or broad-spectrum solar UV. These observations were interpreted to mean that NUV blocks the functioning of the *recA* protease. The conclusion was that the *recA*$^+$ function is not important in the repair of or protection against NUV damage and, by extrapolation, H_2O_2 in exponentially growing *E. coli* cells. The evidence just described is ambiguous as far as a possible role for SOS functions in the repair of or protection against NUV wavelengths.

Renewed interest in a role for H_2O_2 in NUV effects developed when it was demonstrated that pretreatment of *E. coli* cells with sublethal concentrations of H_2O_2 resulted in protection against inactivation by lethal concentrations of H_2O_2 (8) as well as broad-spectrum NUV (33, 39). If H_2O_2 is an important product resulting from NUV treatment of *E.*

coli, one might expect that catalase-deficient mutants would be sensitive to NUV inactivation. We have shown that strain RT10 (*nur*$^+$) carrying an insertion mutation in the *katF* gene was sensitive to inactivation by both broad-spectrum NUV and H_2O_2 (Fig. 4 and 5). When the *katF* insertion mutation was transduced into a strain carrying the *nur* mutation (RT8), the NUV sensitivity of the recipient to NUV or H_2O_2 inactivation was unaltered (Fig. 6 and 7). These results suggested that *nur* might be a mutant allele of the *katF* gene. The results of the transduction experiments (Table 2) were consistent with this idea, if it is assumed that strain KL16-99 contains an inversion in which *katF* (*nur*) has been displaced toward min 50 away from the *cysC*$^+$ gene (min 59.2). In previous experiments (37), it was found that of 247 *cysC*$^+$ transductants, none proved to be sensitive to NUV, while 9 were *recA1* (FUV sensitive). If *katF* were at min 58.9, as suggested by Loewen et al. (21a), then a substantial fraction of the *cysC*$^+$ transductants should have been sensitive to broad-spectrum NUV inactivation (~30%). Since strain RT7h is derived ultimately from RT2 (20, 35, 37), the recombinational event giving rise to RT2 (and ultimately the other RT strains carrying the *nur* allele) from the mating of AT713 (F$^-$) and KL16-99 (Hfr) must have resulted in a restoration of the inverted segment to its proper orientation (37) (see Table 2 of this paper for recombinant frequencies suggesting a possible inversion). Therefore, when the transducing bacteriophage was prepared on either strain RT2 or RT7h, *cysC*$^+$ transductants of AT713 had a significant probability (20 to 30%) of being cotransduced to *nur* (*katF*). Consistent with the hypothesis that *nur* is an allele of *katF* is the absence of catalase (HP-II) activity in extracts of cells designated *nur* (RT7 and RT8) or *katF* (RT8 *katF* and RT10 *katF*) as visualized on polyacrylamide gels (Fig. 9). The transduction experiments (Table 2) and mating experiments (37) strongly suggest that KL16-99 has an inverted segment in the area of the genome involving the *katF* gene. This alteration might modify the regulation of the gene in vivo so that protection against NUV inactivation is not afforded but activity of the HP-II enzyme can be demonstrated in vitro (Fig. 9, lane L).

Another possible explanation for the NUV sensitivity of strain KL16-99 is that this sensitivity is controlled by a second gene unrelated to *nur* (*katF*). The major difficulty with this hypothesis is that strains RT2 and RT7h were ultimately derived from the mating of KL16-99 (*nur*) with AT713 (*nur*$^+$). Although the distribution of recombinants from this mating (37) does not exclude absolutely the possibility of a second gene controlling NUV sensitivity, the inversion hypothesis requires less complex assumptions than does a second-gene hypothesis.

Loewen et al. (23) have shown that both *katE* and *katF* control the activity of the *E. coli* catalase (HP-II). The *katG* gene controls the activity of the peroxidase (HP-I). If both *katE* and *katF* code for subunits of a single enzyme, it seems paradoxical that the *katE* mutation does not sensitize *E. coli* cells to inactivation by NUV or H_2O_2 (Fig. 4 and 5), while *katF* results in sensitization. There are at least two possibilities to explain this. (i) The *katF* gene may specify a subunit of the HP-II enzyme concerned with enzyme activity (22), while *katE* may control a regulatory subunit of the enzyme. Since the experiments described here involved stationary-phase cells, it is possible that HP-II may not be subject to the regulation, which could be specific to exponentially growing cells. (ii) Assuming that the *katE* gene controls a regulatory subunit of HP-II, the mutation in the *katE* gene may prevent activity in vitro (activity gels) while allowing activity in vivo.

It appears that the *katG* mutation in an *nur* (*katF*) background sensitizes cell to NUV inactivation, while not appreciably altering sensitivity to H_2O_2 (Fig. 6 and 7). This result might mean that in a *katF*$^+$ background the HP-I enzyme specified by *katG*$^+$ does not play an important role in the elimination of NUV-generated H_2O_2. When HP-II activity is eliminated by mutations in the *katF* gene, the NUV-generated H_2O_2 may be a substrate for HP-I (*katG*$^+$; Fig. 6 and 7). Thus, the *katG* gene product is essential for defense against oxidative damage only when the major defense provided by the *katF* gene product is absent (as in RT8 *katG*; Fig. 9, lane I). That RT8 *katG* can survive NUV or H_2O_2 at all, in spite of being completely catalase deficient, suggests that other genes are involved in defense against oxidative damage in *E. coli*. Christman et al. (4) suggest that at least 30 proteins are induced in response to oxidative damage.

When stationary-phase *E. coli* cells were treated with 50 mM H_2O_2, the inactivation kinetics were initially exponential for cell populations carrying the *nur* (*katF*) mutation (Fig. 1). It appears that the sensitivity of stationary-phase cells to inactivation by H_2O_2 is independent of the *recA1* or *uvrA6* mutation, as was true for inactivation by broad-spectrum NUV (35, 37). The initial exponential slope in the inactivation curves for *nur* (*katF*) strains was followed by a tail (Fig. 1). One possible explanation for the tailing in these curves is that the enzymatic activity of the cells in suspension breaks down reagent H_2O_2. Thus, the concentration of H_2O_2 in the suspension decreases and allows the cells to survive. This problem was eliminated by using an H_2O_2-generating system (19). This system ensures a relatively constant concentration of H_2O_2 throughout the experiment. Note that the inactivation kinetics of *nur* (*katF*) cells subjected to this system were essentially exponential (Fig. 2, 5, and 7). In addition, a second portion of H_2O_2 added to a suspension of cells treated with a single dose of H_2O_2 resulted in the restoration of exponential inactivation kinetics (Fig. 3).

This work demonstrates that *nur* and *katF* are alleles of a gene whose product (HP-II) is essential for protection against oxidative damage encountered during exposure to H_2O_2 or NUV. These results add support to the general notion that one of the products of NUV treatment of cells is H_2O_2 (31–33). The observations reported here support the general conclusion that the mechanisms which underlie FUV and NUV mutagenesis and lethality are fundamentally different. The effects of FUV are based on direct absorption by DNA, while NUV effects result from the absorption of NUV wavelengths by endogenous photosensitizers (probably porphyrins [38a]) and the formation of oxygen-related radicals (18).

ACKNOWLEDGMENTS

This work was supported by a grant from the National Science Foundation (Genetic Biology Panel; PCM 831-5595). L.J.S. wishes to express her appreciation for support in the form of a research assistantship provided by the School of Life Sciences, University of Illinois (Urbana-Champaign).

We thank F. S. Jacobson (University of California, Berkeley) for his advice on the staining procedure.

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