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Identification and Characterization of Hydrogen Peroxide-Sensitive Mutants of *Escherichia coli*: Genes That Require OxyR for Expression

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Escherichia coli **produces an inducible set of proteins that protect the cell from exogenous peroxide stress. A subset of these genes is induced by hydrogen peroxide and is controlled at the transcriptional level by the OxyR protein. To identify additional genes involved in protection from hydrogen peroxide, a library of random transcriptional fusions of λp*lac*Mu53 was screened for hydrogen peroxide sensitivity and 27 such mutants were identified. These fusions were transduced into nonlysogenic strains to ensure that the phenotypes observed were the result of a single mutation. The mutants were grouped into three classes based on the expression of the *lacZ* fusion during growth in *oxyR*$^+$ and Δ*oxyR* backgrounds. The expression of the *lacZ* fusion in 8 mutants was independent of OxyR, 10 mutants required OxyR for expression, and 6 mutants showed reduced levels of expression in the presence of OxyR. OxyR dependence varied from 2- to 50-fold in these mutants. The OxyR-dependent phenotype was complemented by a plasmid-borne copy of *oxyR* gene in all mutants. Three mutants exhibited dual regulation by OxyR and RpoS. We sequenced the fusion junctions of several of these mutants and identified the genetic loci responsible for the hydrogen peroxide-sensitive (*hps*) phenotype. In this study, we report the identification of several genes that require OxyR for expression, including *hemF* (encoding coproporphyrinogen III oxidase), *rcsC* (encoding a sensor-regulator protein of capsular polysaccharide synthesis genes), and an open reading frame, *f497*, that is similar to arylsulfatase-encoding genes.**

All aerobic respiring organisms require protection from reactive oxygen species (including superoxide anion, hydroxyl radical, and hydrogen peroxide) formed from the partial reduction of molecular oxygen to water during oxidative metabolism. Bacterial cells encounter endogenous hydrogen peroxide produced from the dismutation of superoxide or hydroxyl radical as a product of the respiratory chain when oxygen is used as the terminal electron acceptor. In addition, enteric bacteria, such as *Salmonella typhimurium* and *Escherichia coli*, encounter toxic levels of hydrogen peroxide produced by macrophages during engulfment (17). *E. coli* and *S. typhimurium* possess several enzymes that prevent oxidative damage (alkyl hydroperoxidase, catalases, superoxide dismutase, and glutathione reductase) and repair DNA lesions resulting from oxidative damage (e.g., exonuclease III, RecBC nuclease, and endonuclease III) (for a review, see reference 16). It has been shown that both *E. coli* (14) and *S. typhimurium* (49) become resistant to killing by hydrogen peroxide when pretreated with a nonlethal dose (60 μM) of hydrogen peroxide. The adaptation results in the transient accumulation of a distinct group of proteins (10, 28). There are 30 proteins that exhibit elevated level of synthesis, of which 12 proteins are induced immediately after the hydrogen peroxide challenge and 18 proteins are expressed 10 to 30 min after hydrogen peroxide treatment (10). The induction of 9 of the 30 proteins is under positive control of the *oxyR* gene product (10, 28), OxyR, a member of the LysR family of transcriptional regulators (11). Genes known to be induced by OxyR in the presence of sublethal doses of hydrogen peroxide include *katG* (encoding HPI catalase) (10, 28, 39), *ahpCF* (encoding alkyl hydroperoxidase reductase) (10, 28, 39), *gorA* (encoding glutathione reductase) (21), *dps* (a nonspecific DNA-binding protein with a protective function) (3), and *oxyS* (encoding a small untranslated RNA with a probable regulatory function) (21, 22). OxyR protein also represses its own synthesis (10, 39, 42) as well as that of the Mu phage *mom* gene (6). Other members of the OxyR regulon which are overexpressed in a constitutive *oxyR* mutant are yet to be identified. An OxyR-independent hydrogen peroxide-inducible gene product has been identified as DnaK protein (encoded by *dnaK* gene), which is involved in DNA biosynthesis (28). Some proteins induced under hydrogen peroxide stress are also induced by other forms of stress, including those generated by heat, ethanol, nalidixic acid, and cumene peroxide (28).

To identify members of the hydrogen peroxide (oxidative stress)-induced regulon that are responsible for protecting the cell from oxidative damage, a previously reported (36) random library of transposon-generated *lacZ* operon fusions in *E. coli* (λp*lac*Mu53 fusion phage) was screened for sensitivity to hydrogen peroxide and 26 mutants that showed a hydrogen peroxide-sensitive (*hps*) phenotype toward 1.0 mM hydrogen peroxide (on plate assays) were isolated. Here we present the characterization of the *hps* genes that require OxyR for their expression. The identities of these OxyR-activated *hps* genes are reported on the basis of the DNA sequence of the fusion junctions that map to known regions of the *E. coli* chromosome, thereby identifying some of the genes responsible for producing an Hps phenotype.

MATERIALS AND METHODS

Bacterial strains, bacteriophages, and plasmids. All bacterial strains, bacteriophages, and plasmids used in this study are listed in Table 1.

Chemicals and enzymes. All chemicals were obtained from commercial sources. Stock solutions of antibiotics and other nonautoclavable solutions were filter sterilized (pore size, 0.45 μm; Gelman Sciences, Ann Arbor, Mich.) prior to use.

Media used and growth conditions. The rich medium used was Luria-Bertani (LB) broth (10 g of tryptone, 5 g of yeast extract, 10 g of NaCl) adjusted to pH 7.0. For preparation and determination of the titers of P1*vir* lysates, cultures were grown overnight at 37°C with 5 mM $CaCl_2$. The P1-mediated transductions were performed by the method of Miller (27). Solid medium was prepared by

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

Strain(s), phage, or plasmid	Genotype	Source and/or reference
Strains		
MC4100	F$^-$ *araD139* Δ(*argF-lac*)*U169 rpsL150 relA deoC1 ptsF25 rbsR flb5301*	G. Weinstock
GC4468	F$^-$ Δ*lacU169 rpsL*	Laboratory collection
KL765	F$^-$ *lacZ813 lac13 pro met his trp rpsL thi* λ*ind*	K. B. Low
HS008	Same as KL765 but with ϕ80	Laboratory collection
HS6682	Same as HS008 but *uvrD*::Tn*5*	Laboratory collection
RK4936	*araD139* Δ(*argF-lac*)*205 flb5301* non-*gyrA219 relA1 rpsL150 btuB*::Tn*10*	10
TA4112	Same as RK4936 but *oxy*Δ*3*[*oxy*(*oxyR-btuB*)*3*]	10
TA4484	Same as TA4112 but has pMC7 (*lacI*^{q})	21
GC202	Same as GC4468 but *katG17*::Tn*10*	Laboratory collection
GC122	Same as GC4468 but *rpoS13*::Tn*10*	Laboratory collection
NC202	Same as GC4468 but Φ(*katE*::*lacZ*$^+$)*131 katG17*::Tn*10*	Laboratory collection
NC4468	Same as GC4468 but Φ(*katE*::*lacZ*$^+$)*131*	Laboratory collection
HS701 to HS727	Same as MC4100 but Φ(*hps-1*::*lacZ*$^+$) to Φ(*hps-27*::*lacZ*$^+$)	This study
HS701R to HS727R	Same as HS701 to HS727 but *rpoS13*::Tn*10*	P1(GC122) × HS701 to HS727→Tet^r
GC701 to GC727	Same as GC4468 but Φ(*hps-1*::*lacZ*$^+$) to Φ(*hps-27*::*lacZ*$^+$)	This study
RK701 to RK727	Same as RK4936 but Φ(*hps-1*::*lacZ*$^+$) to Φ(*hps-27*::*lacZ*$^+$)	This study
TA701 to TA727	Same as TA4112 but Φ(*hps-1*::*lacZ*$^+$) to Φ(*hps-27*::*lacZ*$^+$)	This study
Phages		
λp*lac*Mu53	λ*imm 'trp' 'lacZ*$^+$ *lacY*$^+$ *lacA' 'uvrD' Xho*::*kan c*Its62 *ner*$^+$ *A*$^+$*'S*	G. Weinstock
P1*vir*		Laboratory collection
Plasmids		
pAQ24	*katG* promoter fused to a promoterless *lacZ*	G. Storz (43)
pAQ25	*oxyR* gene cloned in pKK177-3 with *tac* promoter	G. Storz (21)
pMC7	Plasmid containing *lacI*^{q} gene	G. Storz (21)

adding 15 g of agar per liter of liquid medium. The soft top agar was prepared by adding 7.5 g of agar per liter of liquid medium. For titration of λ phage, the host strain was grown with 10 mM $MgSO_4$ and 0.2% maltose.

Single-colony isolates were inoculated into growth medium supplemented with appropriate antibiotics and incubated overnight at 37°C. All cultures were grown in flasks at a culture/flask volume ratio of 1/5 to ensure good aeration. Growth was monitored by measuring optical density at 600 nm (OD_{600}) with a Shimadzu UV1201 UV-VIS spectrophotometer. For all induction experiments, overnight cultures were serially subcultured twice to an OD_{600} of 0.2 before inoculation into fresh media to an initial OD_{600} of 0.05 to 0.10 or as indicated.

Identification of hydrogen peroxide-sensitive mutant strains. A collection of more than 5,000 random *lacZ* operon fusions to chromosomal promoters were isolated by infecting strain MC4100 with λp*lac*Mu53 (transcriptional fusion phage) as described previously (36, 37). Cultures of individual colonies were grown in 96-well microtiter plates, replica plated onto LB plates and LB plates containing 1.0 mM hydrogen peroxide, and grown overnight at 37°C. Colonies that exhibited poor growth in the presence of hydrogen peroxide were presumptive hydrogen peroxide-sensitive mutants. These presumptive mutants were further tested for catalase activity by flooding the replicates with 30% hydrogen peroxide. Eight hydrogen peroxide-sensitive mutants also exhibited reduced bubbling in the presence of hydrogen peroxide and thus had reduced catalase activity. P1*vir* lysates were prepared (27) on the hydrogen peroxide-sensitive mutants, and the *lacZ* fusion was transduced into strains GC4468 and MC4100 to ensure that only one fusion was present in each mutant derivative. These transductants were tested for hydrogen peroxide sensitivity. The same P1*vir* lysates were also used to transduce the fusions into strains RK4936 (*oxyR*$^+$) and TA4112 (Δ*oxyR*).

Cell survival assays. Six single-colony isolates from each bacterial strain were inoculated into a 96-well microtiter plate containing 0.1 ml of LB per well and incubated at 37°C until saturation. The cultures were replica plated (in duplicate) onto LB agar and LB agar containing 1.0 mM hydrogen peroxide and incubated overnight at 37°C. Relative sensitivity was determined by comparing the growth of mutant strains with those of appropriate parental strains.

To quantitate the survival of cultures exposed to hydrogen peroxide, overnight cultures were serially subcultured twice to an OD_{600} of 0.20 (approximately 3×10^8 cells/ml). The second subculture of each strain was divided into two portions. One portion of the culture was challenged with 1.0 mM hydrogen peroxide (final concentration), while the other served as a control. The cultures were incubated for 60 min at 37°C and 200 rpm. Aliquots of cultures were withdrawn at the indicated times, appropriately diluted, plated onto LB plates in duplicate, and incubated overnight at 37°C. The relative survival was assessed by determining the viable count of treated cultures in relation to that of untreated cultures incubated in parallel. A concentration of 1 mM hydrogen peroxide was used to challenge the growing cultures because this concentration causes "mode-one" killing in *E. coli* that is dependent on cellular metabolism (19). For determining the zone of inhibition, cultures were grown to an OD_{600} of 0.8 and a 200-μl aliquot was plated onto an LB plate with 3 ml of soft agar. The overlaid plates were allowed to dry at room temperature for 30 min. A Whatman (number 3) filter disc (diameter, 7 mm) soaked with 10 μl of 30% hydrogen peroxide was placed on the middle of each overlaid plate and incubated at 37°C, and the diameter of the zone of inhibition of bacterial growth was measured after 12 h.

Enzymatic assays. β-Galactosidase assays were performed by the method of Miller (27). Activities were normalized with respect to culture density and were expressed in Miller units (27).

Plasmid isolation and transformation. Plasmids were isolated from overnight cultures grown in media containing appropriate antibiotics by the alkaline lysis method (35). Competent cells were prepared by the calcium chloride method (35) and were transformed with a total of 50 ng of either plasmid pAQ25 or pMC7 (21, 22). Transformants were selected on LB plates containing 100 μg of ampicillin per ml (pAQ25) or 15 μg of tetracycline per ml (pMC7). For double transformation, 250 ng of total DNA (125 ng of each plasmid) was used. The double transformants were selected on LB plates containing both ampicillin and tetracycline. Six individual isolates from each transformed mutant strain were inoculated into 96-well microtiter plates along with parent strains and appropriate controls and grown at 37°C to saturation. Microtiter wells were replica plated onto plates supplemented with 50 μg of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) per ml with or without 1 mM isopropyl-thio-D-galactosidase (IPTG) as inducer. The plates were incubated at 37°C for 12 h, and the change in color was examined in the presence and absence of IPTG.

Induction of cultures with IPTG. Overnight cultures were serially subcultured twice in LB containing appropriate antibiotics to an initial OD_{600} of 0.2. Cultures were divided into two equal portions; to one portion, IPTG was added to a final concentration of 1 mM. Cultures were incubated at 37°C at 200 rpm. Aliquots of cultures were removed every 30 min, placed on ice with chloramphenicol (150 μg/ml [final concentration]) to stop further protein synthesis, and assayed for β-galactosidase activity. All assays were performed in duplicate.

Induction of λp*lac*Mu53 lysogens by UV and DNA isolation from lysates. To isolate phage DNA, λp*lac*Mu53 lysogens were induced by UV as described previously (33, 37). An overnight culture from a single-colony isolate was subcultured to an OD_{600} of 0.4 (approximately 3.4×10^8 cells/ml of culture) in 50 ml of LB. The culture was centrifuged at 7,000 × *g* at 4°C for 15 min, resuspended in 10 ml of 10 mM $MgSO_4$, and spread on a petri dish (15-cm diameter). The culture suspension was irradiated uncovered for 6 s by placing it 50 cm below two 15-W germicidal UV lamps (NIS G15T8). Total fluence was approximately 31 J/m^2, as measured with a UVX radiometer (Ultraviolet Light Products, San Gabriel, Calif.) and a 254-nm probe (model no. UVX-25). To the irradiated cell

suspension, 5.0 ml of 3× LL broth (90 g of tryptone, 45 g of yeast extract, 45 g of NaCl, 60 mg [each] of adenine, cytosine, guanine, and thymine per liter, pH 7.25) was added and incubated at 37°C with vigorous shaking (200 rpm) until visible lysis occurred (3 to 5 h). The lysate was transferred to 50-ml polypropylene screwcap tubes (catalog no. 25330-50; Corning Inc., Corning, N.Y.) containing a few drops of chloroform and vortexed vigorously for 1 min before being centrifuged at 3,000 rpm for 20 min at 4°C to remove cell debris. An aliquot of the lysate was appropriately diluted and plated with strain MC4100 as host onto LB plates containing 50 μg (each) of streptomycin and X-Gal per ml (phage titers varied from 10^5 to 10^7 PFU/ml). The phage was pelleted by centrifuging the lysate at 35,000 rpm for 30 min in a Beckman SW41Ti rotor (33). DNA from the pelleted phage was isolated by a previously described method (35).

DNA sequencing of the fusion junctions and analysis. The mutator phage employed in this study, λp*lac*Mu53 (7), is a hybrid phage containing λ and phage Mu segments. To identify chromosomal genes immediately adjacent to the integrated phage, we used a DNA sequencing primer complementary to the Mu C end. The primer, AB3818, was a 25-mer, 5′ CCGGAATAATCCAATGTCCTC CCGG 3′, 30 nucleotides downstream from the Mu C end (33). The phage DNA isolated from UV-induced lysates was purified by using a glass membrane ultrafiltration cartridge (catalog no. 15590-060; Gibco BRL, Burlington, Ontario, Canada) as recommended by the manufacturer. The amount of DNA used in each sequencing reaction was 10 to 100 fmol. Sequencing of the phage templates was performed with *Taq* polymerase (AmpliCycle sequencing kit; Perkin-Elmer, Branchburg, N.J.) by using a 5′-end-labelled primer, AB3818, with [α-^{35}S]thio-dATP (>1,000 Ci/mmol). The sequencing reaction was performed in 25 cycles of 95°C for 30 s, 65°C for 30 s, and 72°C for 60 s with a thermal cycler (GeneE thermal cycler; Techne Inc., Princeton, N.J.) equipped with a heated lid (33). The sequences of the fusion junctions were determined from two independent sequencing reactions performed each time with freshly prepared templates. DNA sequences were examined for homology with the data available in the GenBank database by using the program (2).

RESULTS

Isolation of hydrogen peroxide-sensitive mutants containing chromosomal *lacZ* operon fusions. Transcriptional fusion phage λp*lac*Mu53 (7) was used to obtain a random chromosomal *lacZ* operon fusion bank of over 5,000 operon fusions, as described in Materials and Methods. On screening the fusion bank, 27 mutant strains (in the MC4100 background) which were sensitive to 1.0 mM hydrogen peroxide were isolated. Subsequently, the *lacZ* fusions were transduced into strains GC4468 and MC4100 to ensure that only one fusion was present in each mutant derivative. The genes corresponding to the fusions were designated *hps* (hydrogen peroxide-sensitive phenotype). As shown in Table 2, each of these fusions was sensitive to 1.0 mM hydrogen peroxide and exhibited a larger zone of inhibition than did the wild-type strain (refer to Materials and Methods).

Quantitative cell survival assays were performed on most of the *hps* mutants (data not reported). We report the sensitivities of a set of representative mutants (*hps-2*, *hps-4*, *hps-5*, and *hps-10*) compared to those of mutant *hps-7* in isogenic *oxyR*$^+$ and Δ*oxyR* backgrounds (Fig. 1). Exponentially growing cultures were challenged with 1 mM hydrogen peroxide to determine the effect on viability, as *E. coli* undergoes mode-one killing when exposed to 1 to 2 mM hydrogen peroxide (19). At this concentration of hydrogen peroxide, additional cellular responses (apart from the known OxyR-dependent, SOS-mediated, or superoxide-inducible response) that are as yet uncharacterized are induced (19). All the *hps* mutant strains were sensitive (3- to 10-fold) to 1.0 mM hydrogen peroxide. The *hps*Δ*oxyR* double mutants were more sensitive than were the *hps* mutant strains (data not reported). The *hps-7*Δ*oxyR* double mutant exhibited a synergistic hypersensitive phenotype and had an approximately 50-fold-reduced viability compared to that of the wild-type strain, indicating that other genes (such as *hps-7*) may be involved in hydrogen peroxide stress response independent of OxyR.

Effect of OxyR on β-galactosidase expression of *hps*::*lacZ* fusions. Hydrogen peroxide treatment induces the synthesis of 30 proteins in *S. typhimurium* (28). The synthesis of nine of these proteins, including HPI hydroperoxidase, is regulated by OxyR (10, 28), a transcriptional activator (39, 42). Since we isolated 27 *hps* mutants, it was possible that some of the fusions were in OxyR-regulated genes. To determine the effect of OxyR on the expression of various *hps*::*lacZ* mutants, the fusions were transduced into isogenic *oxyR*$^+$ (strain RK4936) and Δ*oxyR* (strain TA4112) backgrounds. However, we were unable to obtain viable transductants for fusion *hps-17*::*lacZ* in an Δ*oxyR* background. All of the transductants were sensitive to hydrogen peroxide in an *oxyR*$^+$ background compared to the parent strain (Table 2), as determined by the radius of inhibition.

As an initial screen, overnight cultures of *hps*::*lacZ* fusions in *oxyR*$^+$ and Δ*oxyR* backgrounds were assayed for β-galactosidase activity to identify OxyR-dependent fusions (Table 3). The mutants could be separated into the following three major classes: OxyR independent (the *oxyR*$^+$/Δ*oxyR* ratio, ≅1); OxyR

TABLE 2. Sensitivities of *hps* mutants to hydrogen peroxide[a]

Strain	Allele	Sensitivity to hydrogen peroxide (mM)[b]		Radius of inhibition (mm)[c]	
		0.0	1.0	*oxyR*$^+$	Δ*oxyR*
RK4936	Wild type	+	+	7.5	15.5[e]
GC4468	Wild type	+	+	8.0	
NC4468	*katE*::*lacZ*	+	±	9.2	
GC202	*katG*::Tn*10*	+	−	11.5	
NC202	*katE*::*lacZ*, *katG*::Tn*10*	+	−	19.5	
HS701	*hps-1*	+	−	10.3	17.3
HS702	*hps-2*	+	−	10.8	22.2
HS703	*hps-3*	+	−	9.5	20.8
HS704	*hps-4*	+	−	12.5	18.7
HS705	*hps-5*	+	−	10.8	20.2
HS706	*hps-6*	+	−	9.0	18.8
HS707	*hps-7*	+	−	11.3	24.0
HS710	*hps-10*	+	−	12.2	21.2
HS711	*hps-11*	+	−	12.5	15.8
HS712	*hps-12*	+	−	8.8	18.8
HS713	*hps-13*	+	−	11.3	17.5
HS714	*hps-14*	+	−	8.8	17.8
HS715	*hps-15*	+	−	11.5	21.8
HS716	*hps-16*	+	−	9.3	19.0
HS717	*hps-17*	+	−	9.0	NA[d]
HS718	*hps-18*	+	−	8.7	20.2
HS719	*hps-19*	+	−	8.8	19.7
HS720	*hps-20*	+	−	8.8	17.0
HS721	*hps-21*	+	−	10.5	17.2
HS722	*hps-22*	+	−	8.7	16.8
HS723	*hps-23*	+	−	11.7	18.2
HS724	*hps-24*	+	−	9.0	21.2
HS725	*hps-25*	+	−	9.7	19.3
HS726	*hps-26*	+	−	9.3	19.3
HS727	*hps-27*	+	−	8.8	16.8

[a] Single-colony isolates were inoculated into 96-well microtiter plates, grown at 37°C to saturation, and replica plated onto LB plates and LB plates containing 1.0 mM hydrogen peroxide, as described in Materials and Methods. In *hps-8* and *hps-9* mutants, the fusion junction was in the *katE* gene; hence, they were not included in this table or subsequent studies.

[b] +, growth; ±, slight growth; −, no growth.

[c] A 0.2-ml aliquot of a culture grown for 6 h was plated on an LB plate with 3 ml of LB soft agar. A filter disc (diameter, 7 mm) with 10 μl of 30% hydrogen peroxide was placed in the middle of each overlaid petri dish and incubated for 12 h at 37°C. Data are averages of triplicate plate assays. The standard error for each set of experiments was less than 0.2.

[d] NA, not available. The *hps-17*::*lacZ* fusion could not be transduced into an Δ*oxyR* background.

[e] Radius of inhibition for isogenic Δ*oxyR* strain TA4112.

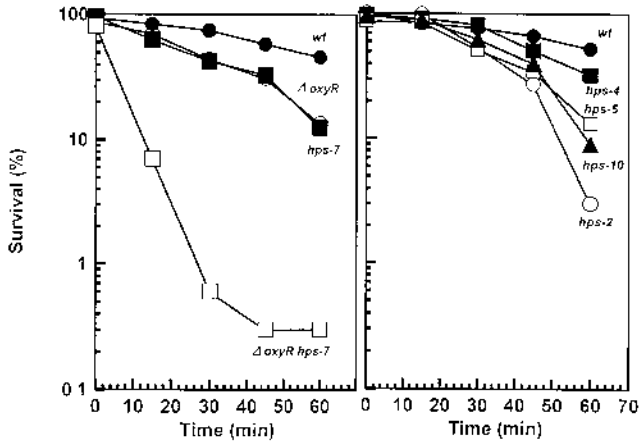

FIG. 1. Effect of 1 mM hydrogen peroxide on survival of *hps* mutants. Exponentially growing cultures were challenged with 1 mM hydrogen peroxide and assayed for survival over a period of 60 min, as described in Materials and Methods. The wild-type (*wt*) strain was RK4936, and the Δ*oxyR* strain was TA4112. The mutant strains exposed to the challenge were HS702 (*hps-2*::*lacZ*), HS704 (*hps-4*::*lacZ*), HS705 (*hps-5*::*lacZ*), HS707 (*hps-7*::*lacZ*), and HS710 (*hps-10*::*lacZ*).

activated, showing a decreased level of β-galactosidase expression of an *hps*::*lacZ* fusion in an *oxyR* deletion background (*oxyR*$^+$/Δ*oxyR* ratio, >2); and OxyR repressed, showing a higher level of *lacZ* expression in an *oxyR* deletion background (*oxyR*$^+$/Δ*oxyR* ratio, <0.5).

Effect of OxyR on growth phase-dependent expression of *hps*::*lacZ* fusions. OxyR can act as a transcriptional activator (39, 42, 43) and a repressor, repressing its own expression (10) and that of the *mom* gene of phage Mu (6). Since the role of OxyR has been studied using exponential-phase cultures (10, 28, 29) and the differences in expression of the *hps*::*lacZ* fusions in overnight cultures were modest between *oxyR*$^+$ and Δ*oxyR* backgrounds for most fusions (Table 3), we decided to study the expression of the *hps*::*lacZ* fusions in isogenic *oxyR*$^+$ and Δ*oxyR* backgrounds during normal growth to determine which of these *hps* loci are activated or repressed by OxyR. Each of these *hps*::*lacZ* operon fusions showed individual variable expression patterns during growth, indicating that each fusion is unique in its regulation. Depending on the pattern of *lacZ* expression, the fusions were grouped into one of three classes (Table 4). The *hps*::*lacZ* fusions whose levels were independent of the status of OxyR were assigned to class I (*hps-1*, *hps-3*, *hps-7*, *hps-11*, *hps-12*, *hps-15*, *hps-18*, and *hps-23*) (Fig. 2A). Ten mutants were grouped as class II fusions because they were OxyR activated (required functional OxyR for maximal expression of the *lacZ* fusion; *hps-2*, *hps-4*, *hps-5*, *hps-10*, *hps-14*, *hps-16*, *hps-20*, *hps-21*, *hps-24*, and *hps-27*). A representative member of this group, *hps-21*::*lacZ*, showed growth phase-dependent expression in an *oxyR*$^+$ background and had almost 60-fold-higher activity than that of an isogenic Δ*oxyR* strain (Fig. 2B). All other members of this group exhibited 5- to 50-fold-higher expression of the fusion in an *oxyR*$^+$ background than in an Δ*oxyR* background. Unlike in class II mutants, in class III mutants the expression of the *lacZ* fusion was repressed by a functional OxyR protein. During exponential phase of growth, the expression of these fusions was 2- to 10-fold lower in the isogenic *oxyR*$^+$ background compared to that in the Δ*oxyR* background (data not shown). The maximum difference in expression was significant enough to classify these fusions as OxyR-repressed genes (*hps-6*, *hps-13*, *hps-19*, *hps-22*, *hps-25*, and *hps-26*). A representative member of this class of mutant is *hps-19* (Fig. 2B). The class III mutants will be discussed in a subsequent communication.

TABLE 3. Effect of OxyR on the expression of *hps* fusions[a]

Fusion	β-Galactosidase activity (Miller units)		*oxyR*$^+$/Δ*oxyR* ratio
	oxyR$^+$	Δ*oxyR*	
hps-1::*lacZ*	16.7	40.4	0.4
hps-2::*lacZ*	92.0	67.0	1.4
hps-3::*lacZ*	89.3	88.3	1.0
hps-4::*lacZ*	67.3	12.0	5.6
hps-5::*lacZ*	18.8	14.1	1.3
hps-6::*lacZ*	8.0	15.2	0.5
hps-7::*lacZ*	1.1	1.2	0.9
hps-10::*lacZ*	97.0	80.1	1.2
hps-11::*lacZ*	89.3	88.9	1.0
hps-12::*lacZ*	18.7	10.7	1.7
hps-13::*lacZ*	1.2	43.7	0.03
hps-14::*lacZ*	30.1	18.5	1.6
hps-15::*lacZ*	36.8	37.3	1.0
hps-16::*lacZ*	53.1	15.1	3.6
hps-18::*lacZ*	95.0	83.4	1.1
hps-19::*lacZ*	6.7	43.4	0.2
hps-20::*lacZ*	63.0	13.0	4.8
hps-21::*lacZ*	65.8	4.7	14.0
hps-22::*lacZ*	21.3	51.7	0.4
hps-23::*lacZ*	17.0	23.2	0.7
hps-24::*lacZ*	54.0	17.5	3.1
hps-25::*lacZ*	60.2	84.5	0.7
hps-26::*lacZ*	79.3	96.5	0.8
hps-27::*lacZ*	60.9	12.6	4.8

[a] Overnight cultures grown in LB were assayed for β-galactosidase activity.

Complementation of *hps* mutants by a plasmid-borne *oxyR* gene. As some of the *hps*::*lacZ* mutants exhibited OxyR-dependent expression, a plasmid-borne copy of the *oxyR* gene (21) was used to confirm the phenotype by complementing the expression of the *hps*::*lacZ* fusion in an Δ*oxyR* background. All *hps* mutants in the TA4112 background (Δ*oxyR*) were transformed with plasmids pAQ25, containing an IPTG-inducible *oxyR* construct (p*tac-oxyR*), and pMC7 (*lacI*^{q}) (21). The double transformants were replica plated onto X-Gal-containing plates with and without 1 mM IPTG (as described in Materials and Methods) to confirm complementation. Subsequently, expression assays were performed with cultures as described in Materials and Methods. The results (Table 5) indicate that all class II mutants were complemented by the *oxyR* gene and the degree of complementation varied from 2- to 14-fold. Previous efforts to complement these mutants with a plasmid construct of the *oxyR* gene containing wild-type OxyR resulted in modest levels of complementation, probably due to the autoregulatory effect of OxyR (data not shown). All class III mutants were also complemented by OxyR with respect to the repression phenotype (data not shown).

Induction of *hps*::*lacZ* fusions with 60 μM hydrogen peroxide. Hydrogen peroxide induces the synthesis of 30 proteins in *S. typhimurium*, of which 9 are regulated by OxyR (10, 28). Since the mutations in *hps* genes conferred a hydrogen peroxide-sensitive phenotype and since some of the *hps*::*lacZ* fusions appeared to be activated by OxyR, we tested whether these *hps*::*lacZ* fusions were hydrogen peroxide inducible (i.e., exhibited increased expression in presence of 60 μM hydrogen peroxide) in accordance with the known function of OxyR protein. Seven OxyR-activated fusions exhibited some degree of inducibility in the presence of 60 μM hydrogen peroxide (Table 6). A plasmid construct carrying a *katG* promoter fused

TABLE 4. Classification of *hps*::*lacZ* mutants

Class	Characteristic(s)	Fusions	Known mutants with similar phenotypes[a]
I	Independent of OxyR	*hps-1*, *hps-3*, *hps-7*, *hps-11*, *hps-12*, *hps-15*, *hps-18*, *hps-23*	*sodA*, *sodB*, *xthA*, *dnaK*
II	OxyR activated; requirement of functional OxyR for maximum expression	*hsp-2*, *hps-4*, *hps-5*, *hps-10*, *hps-14*, *hps-16*, *hps-20*, *hps-21*, *hps-24*, *hps-27*	*katG*, *ahpCF*, *gorA*, *dps*, *oxyS*
III	OxyR repressed; expression of the fusion reduced in the presence of OxyR	*hps-6*, *hps-13*, *hps-19*, *hps-22*, *hps-25*, *hps-26*	*oxyR*, *mom* (ΦMu)

[a] See text for discussion and references.

to a promoterless *lacZ* gene (43) was transformed into an *oxyR*$^+$ strain (RK4936). Its induction was used to measure OxyR activation and served as a control. There was 1.5- to 5-fold induction of various *hps*::*lacZ* fusions in the presence of 60 μM hydrogen peroxide (Table 6). The *hps-16*::*lacZ* fusion exhibited fivefold induction, while *hps-21*::*lacZ* exhibited modest twofold induction.

Regulation of *hps* loci by RpoS. The expression of various *hps*::*lacZ* fusions during entry into stationary phase (Fig. 2) suggests that these loci are regulated by RpoS, a stationary-phase-dependent sigma factor (24, 26, 41). The β-galactosidase activities in these mutants increased sharply as cultures entered the stationary phase of growth, as shown in the case of *hps-21*::*lacZ* (Fig. 3). Since it is known that the expression of *katG* (23, 29), *dps* (3), and *gorA* (4) genes is regulated by both OxyR (in a hydrogen peroxide-inducible manner) and RpoS in a growth phase-dependent manner, we wanted to determine whether any of the *hps* genes were also regulated by RpoS. An *rpoS*::Tn*10* allele was transduced from strain GC122 into all the *hps* mutants in the MC4100 background. The transductants were screened for reduced β-galactosidase activities by replica plating onto X-Gal-containing plates, as described in Materials and Methods. Five *rpoS hps* double mutants exhibited lower β-galactosidase activities on X-Gal plates than did the isogenic *rpoS*$^+$ strain. The activity levels of the *hps*::*lacZ* fusions in these strains were determined during normal growth, and three of these *hps* genes showed various degrees (2- to 10-fold) of dependence on RpoS (*hps-5*, *hps-19*, and *hps-21*) (Table 7). The *hps-21*::*lacZ* mutant showed more-than-threefold dependence on RpoS, similar to those of some previously known genes, such as *katG* (23, 29), *gorA* (4), and *dps* (3) (Fig. 3).

Identification of some of the *lacZ* fusion junctions by DNA sequencing. To identify *hps* genes mutated by the insertion of λp*lac*Mu53 phage, the integrated phage was induced from cultures and sequenced, as described in Materials and Methods. Here we report the fusion junctions of class II *hps* mutants (OxyR activated) that mapped to previously known genes of *E. coli*, thereby identifying new members of the OxyR regulon (Table 8). The fusion junction in *hps-27* is in a gene encoding a sensor protein of a two-component regulatory pathway of colanic acid capsular polysaccharide synthesis, RcsC (40). The insertion is in a proline codon 671 bases from the initiation of translation (40). The fusion junctions in *hps-5* (*thiG*) (5, 47), *hps-10* (*gldA*) (5, 46), and *hps-24* (*uvrD*) (13, 50) are oriented opposite to the known promoter transcribing the gene. In *hps-24*::*lacZ*, the insertion is in the noncoding strand, 497 bases

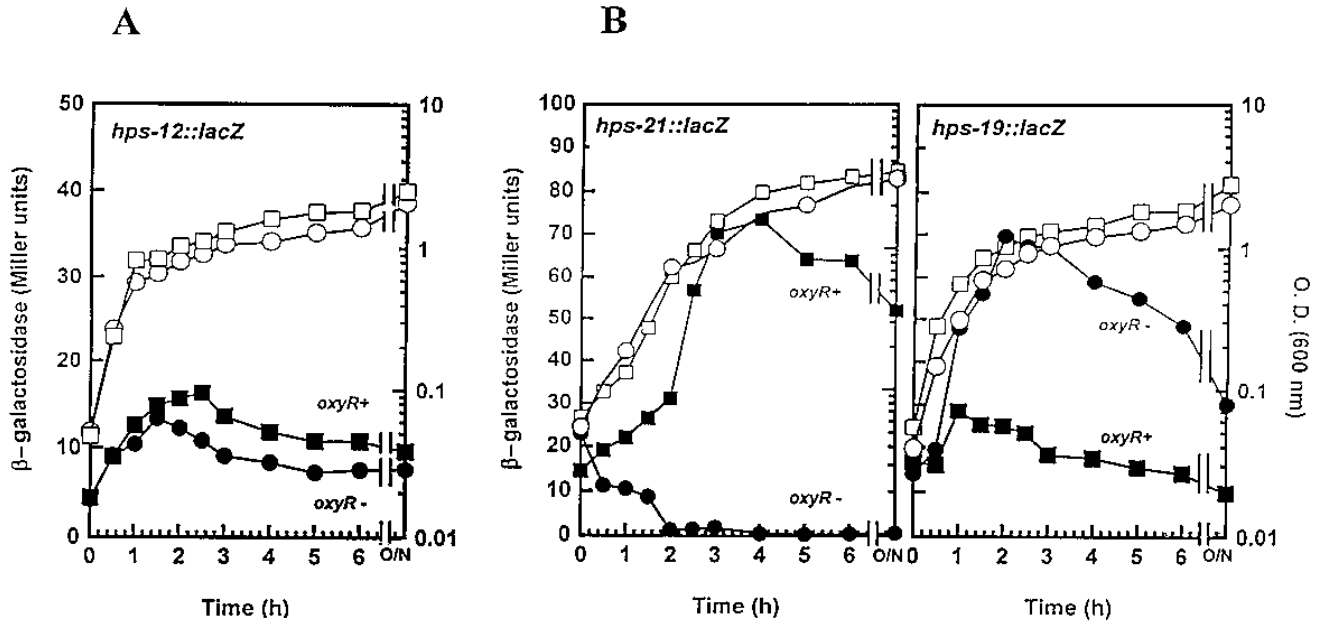

FIG. 2. Expression of *hps*::*lacZ* fusions in wild-type and Δ*oxyR* backgrounds. (A) Representative class I fusion mutant showing an OxyR-independent expression pattern during growth in LB medium in wild-type and Δ*oxyR* backgrounds. (B) OxyR-dependent expression. The expression of the *hps-21*::*lacZ* fusion was very low in the absence of OxyR; hence, this fusion is a class II (OxyR-activated) fusion mutant. *hps-19*::*lacZ* expression was repressed in the presence of OxyR; thus, this fusion is a class III fusion mutant. Symbols: ■ and ●, expression of the *lacZ* fusion in wild-type and Δ*oxyR* backgrounds, respectively; □ and ○, growth (expressed in OD_{600}) for wild-type and Δ*oxyR* strains, respectively. O/N, overnight.

TABLE 5. Complementation of *hps* mutants by a plasmid-borne *oxyR* gene under an IPTG-inducible *tac* promoter[a]

Allele	Class	β-Galactosidase activity (Miller units)				
		Wild type	ΔoxyR			
			Without vector	Plasmids only	pAQ25 (*oxyR*)	
					− IPTG	+ IPTG[b]
OxyR independent, *hps-11*	I	124.0	138.0	139.0	130.0	136.0
OxyR activated						
hps-2	II	109.0	36.6	34.0	64.8	131.5 (3.6)[c]
hps-4	II	60.5	34.4	38.8	63.4	72.8 (2.1)
hps-5	II	40.9	27.6	28.8	21.1	47.4 (1.7)
hps-10	II	103.6	40.5	31.9	84.2	134.1 (3.3)
hps-14	II	78.7	45.9	50.5	87.9	165.8 (3.6)
hps-16	II	91.6	49.3	50.5	77.4	147.5 (3.0)
hps-20	II	65.0	27.2	34.6	96.0	146.4 (5.4)
hps-21	II	59.0	6.0	5.8	20.3	84.7 (14.1)
hps-24	II	35.4	29.1	28.4	62.7	92.0 (3.2)
hps-27	II	105.0	35.1	31.9	84.3	219.0 (6.2)

[a] All cultures were serially subcultured twice to an OD_{600} of 0.2. Cultures containing pAQ25 transformants were divided into two equal portions, and to one portion IPTG was added to a final concentration of 1 mM. Cultures were incubated at 37°C at 200 rpm. Aliquots of cultures were removed periodically to assay β-galactosidase activities. Data are for aliquots taken 90 min after addition of IPTG. Addition of 1 mM IPTG to cultures of wild-type, Δ*oxyR*, and Δ*oxyR*/pKK177-3 (vector only) strains did not alter the basal level of β-galactosidase activity relative to that of an untreated culture. Only one member of the OxyR-independent group has been included here. All other members of this group exhibited similar levels of expression in both $oxyR^+$ and Δ*oxyR* backgrounds.

[b] 1 mM (final concentration).

[c] Fold induction relative to that of an Δ*oxyR* strain under similar conditions.

from the translational start site, disrupting the codon for Val 166 (50). Similarly, in the *hps-5*::*lacZ* mutant, the insertion is in the noncoding strand of the *thiG* gene, disrupting the codon for Ala 146 (47), and in *hps10*::*lacZ*, the insertion is in the noncoding strand, disrupting the codon for Arg 90 (5).

The fusion in *hps-21* is in an open reading frame (ORF), *f497* (8, 38), whose probable translation product is similar to arylsulfatase enzymes and is close to the *oriC* region of the chromosome that initiates replication. Although different promoter elements in this region have been mapped (20), we do not know the ones that are driving the expression of the fusion. The fusion in the *hps-4*::*lacZ* mutant is in the *hemF* gene, which encodes the aerobically produced coproporphyrinogen III oxidase involved in heme biosynthesis (45). The *hps-2* mutation maps to the noncoding strand of cell division gene *ftsJ* (1). However, the bases immediately following the fusion junction in these mutants do not correspond to the published sequence information. The discrepancy in sequence information can be resolved by cloning the fusion junctions by the mini-Mu method (27) and comparing them with the sequences obtained by the UV method (33).

Sensitivity of *hps-24* to mitomycin. Strains with mutations in the *uvrD* gene, whose product is a DNA helicase II involved in nucleotide excision repair and mismatch repair of DNA, are sensitive to mitomycin (an interstrand linking agent causing replication blocks). We compared the sensitivity of strain RK724, containing *hps-24* (*lacZ* inserted in the *uvrD* gene), to

TABLE 6. Effects of hydrogen peroxide on the expression of OxyR-activated and hydrogen peroxide-inducible *hps*::*lacZ* fusions during exponential phase[a]

Strain	*lacZ* fusion	β-Galactosidase activity (Miller units)		Fold induction
		Without H_2O_2	With H_2O_2	
RK4936/pAQ24	*katG*::*lacZ*	1,800	3,300	1.8
RK702	*hps-2*::*lacZ*	100	161	1.6
RK704	*hps-4*::*lacZ*	6.5	11.9	1.8
RK710	*hps-10*::*lacZ*	52.2	135.3	2.5
RK716	*hps-16*::*lacZ*	8.8	43.2	4.9
RK720	*hps-20*::*lacZ*	45.7	68.4	1.5
RK721	*hps-21*::*lacZ*	13.5	23.3	1.8
RK727	*hps-27*::*lacZ*	31.7	88.1	2.8

[a] Overnight cultures of strains were subcultured twice to an OD_{600} of 0.2, divided into two portions, and inoculated into flasks containing prewarmed LB to an initial OD_{600} of 0.1. To one flask, hydrogen peroxide was added to a final concentration of 60 μM; both flasks were incubated for 60 min at 200 rpm and 37°C. After 60 min, cultures were placed on ice, and chloramphenicol (150 μg/ml [final concentration]) was added to stop further protein synthesis. Subsequently, cultures were assayed for β-galactosidase activity.

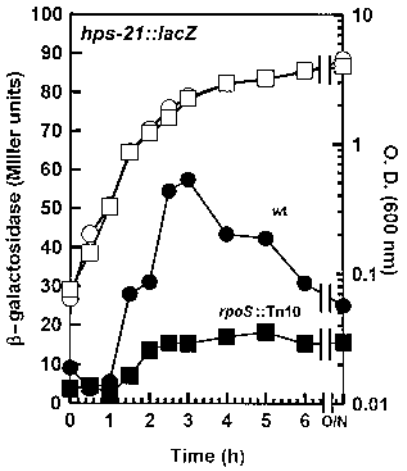

FIG. 3. RpoS-dependent expression of *hps-21*::*lacZ* during growth. The β-galactosidase activities of the *hps-21*::*lacZ* fusion in isogenic wild-type (*wt*) and *rpoS*::Tn*10* backgrounds were determined in LB medium during growth, as described in Materials and Methods. Symbols: ■ and ●, β-galactosidase activities in *rpoS* and wild-type backgrounds, respectively; □ and ○, growth (expressed in OD_{600}) for wild-type and *rpoS* strains, respectively. O/N, overnight.

TABLE 7. Regulation of OxyR-activated genes by RpoS[a]

Allele	Growth phase[b]	β-Galactosidase activity (Miller units)		Ratio
		rpoS$^+$	*rpoS*	
hps-5	Exponential	12.8	16.0	
	Early stationary	50.3	26.1	2
hps-19	Exponential	10.8	7.6	
	Early stationary	37.3	3.8	10
hps-21	Exponential	3.6	4.5	
	Early stationary	54.4	15.4	3.5

[a] Overnight cultures were serially subcultured twice to an OD_{600} of 0.2 and inoculated into LB media to an initial OD_{600} of 0.05. Aliquots of cultures were removed every 30 min and assayed for β-galactosidase activity.

[b] Exponential phase refers to cultures at an OD_{600} of 0.2, growing for 30 min after subculture; early stationary phase refers to cultures at an OD_{600} of 2.0, growing for 2.5 h after subculture.

mitomycin relative to that of another nonisogenic *uvrD* mutant, HS6882 (Table 9). The *hps-24* mutant was sensitive to mitomycin compared to the parent strains, and the degree of sensitivity was similar to those of strains HS008 (wild type) and HS6882 (*uvrD*::Tn*5*) (both sets exhibited a 3-mm increase in the radius of inhibition of the mutant strain).

DISCUSSION

It is known that oxidative stress (hydrogen peroxide treatment) induces the synthesis of many protein in *S. typhimurium* (10, 28). However, only a few of the genes expressing the above-mentioned proteins have been identified. From a random chromosomal fusion library, 26 mutants that exhibited a hydrogen peroxide-sensitive phenotype reproducibly in three different genetic backgrounds (MC4100, GC4468, and RK4936) were isolated. Since transposon insertions were used to generate these fusions, our screen may have missed the identification of genes essential for survival during hydrogen peroxide-stressed conditions (as mutations in these genes will not be viable) or during growth. However, we have identified a set of nonessential genes that play a significant role in cell survival against oxidative damage.

These genes were grouped into three major classes based on expression pattern of the *hps*::*lacZ* fusions during growth, hydrogen peroxide inducibility, and complementation by a plasmid-borne copy of the *oxyR* gene (Table 4). The expression of the *lacZ* fusion of class I mutants was independent of OxyR regulation. A mutation in the *xthA* gene, encoding exonuclease III, which is involved in the repair of damaged DNA, renders the cell sensitive to hydrogen peroxide (15) and is similar to our class I mutants. The hydrogen peroxide- and heat shock-inducible *dnaK* gene product, DnaK, which is involved in the initiation of DNA replication, may play a role in the macromolecular assemblies of proteins under oxidative stress as is evident from its similarity to the *groE* gene product, which is involved in encoding molecular chaperones like GroEL and GroES proteins. Mutants deficient in *dnaK*, like class I *hps* mutants, are hydrogen peroxide sensitive (9).

Class II *hps* genes require functional OxyR protein for maximum expression. Some class II mutants, which are activated by OxyR, showed increased levels of expression at the onset of stationary phase (Fig. 3). It has been recently shown not only that *katG* (23, 29), *dps* (3), and *gorA* (4) are members of the OxyR regulon but that their growth phase expression is regulated by RpoS, the second principal sigma factor responsible for starvation stress-induced gene expression (24, 26, 41). The expression of *katG* (encoding HPI hydroperoxidase) increases to a maximum as the cultures enter into stationary phase and is regulated at the level of transcription by RpoS; however, the hydrogen peroxide inducibility is regulated in exponential-phase cultures at the level of transcription by OxyR (10, 28, 29). We have identified two genes among class II (OxyR-activated) mutants (*hps-5* and *hps-21*) and one among class III mutants (*hps-19*) that show dual regulation.

One of the identified fusions of the OxyR-activated class mapped to *rcsC*, a probable membrane-bound signal transducer of a two-component regulatory network that controls capsular polysaccharide biosynthesis (40). Like mutations affecting other sensor transducers, such as *ntrB* (30), mutations in *rcsC* do not appear to confer a distinguishing phenotype (40). However, it is possible that a cell that is defective in capsule synthesis is vulnerable to some exogenous membrane-damaging agents. This may explain why an *rcsC* mutant, defective in expressing the *cps* capsular polysaccharide biosynthesis genes, is hydrogen peroxide sensitive. The lack of an observable phenotype for *rcsC* mutants may be partially due to an overlap in genetic regulation. For example, cross talk between distinct systems, such as those that regulate nitrogen assimilation and chemotaxis (30), may suppress the effect of a

TABLE 8. Locations of the *lacZ* fusion junctions in OxyR-activated *hps* mutants that map to known *E. coli* genes

Fusion	Chromosomal location (min)	Gene[a]	Function (reference[s])
hps-2	67.4	*ftsJ*[b]	Cell division gene (1)
hps-4	50	*hemF*[c]	Aerobically produced coproporphyrinogen III oxidase; required for synthesis of protoheme IX (45)
hps-5	89	*thiG*[d]	Required for synthesis of thiazole in thiamine synthesis (5, 47)
hps-10	89	*gldA*[e]	Glycerol dehydrogenase (5, 46)
hps-21	81.5	*f497*	Similar to arylsulfatase; unknown function (8, 38)
hps-24	86	*uvrD*[f]	DNA-dependent ATPase and DNA helicase II (13, 50)
hps-27	48	*rcsC*	Sensor-regulator protein of capsular polysaccharide-synthesizing genes (50)

[a] Locations of *lacZ* fusion junctions within known sequences were identified from Blast searches (2).

[b] The insertion is in opposite orientation relative to transcription of the *ftsJ* gene. We are presently unable to identify the potential promoter element driving the expression of the integrated *lacZ* gene from information obtained from GenBank entry ECOUW67.

[c] *E. coli* gene with maximum homology to our sequence data.

[d] The insertion is in opposite orientation from transcription of the *thiG* gene. The potential promoter element driving the transcription of the integrated *lacZ* gene may be that of the *htrC* gene from information obtained from GenBank entry ECOUW89 (5).

[e] The insertion is in opposite orientation from transcription of the *gldA* gene. We are presently unable to identify potential promoter elements driving the expression of the integrated *lacZ* gene from information obtained from GenBank entry ECOUW89 (5).

[f] The insertion is in opposite orientation from transcription of the *uvrD* gene. The potential promoter element driving the transcription of the integrated *lacZ* gene may be that of either ORF *f125* or ORF *f161* from information obtained from GenBank entry X00738 (13).

TABLE 9. Sensitivity of HS724 (*uvrD*::*lacZ*) to mitomycin

Strain	Relevant genotype	Radius of inhibition (mm)[a]
RK4936	Wild type	5.5
HS008	Wild type	7.5
TA4112	Δ*oxyR*	5.5
HS6882	*uvrD*::Tn*5*	10.5
RK724	*uvrD*::*lacZ*	8.2
TA724	Δ*oxyR uvrD*::*lacZ*	8.8

[a] A 0.2-ml aliquot of a culture grown for 6 h was plated on an LB plate with 3 ml of soft agar. Filter discs containing mitomycin (10 μl from a 2-mg/ml stock) were placed on the middle of each overlaid plate and incubated for 12 h at 37°C. Each value is the average of triplicate assays. The standard error for each set of experiments was less than 0.2 mm.

mutation that affects a given two-component regulator. Determinations of levels of catalase expression, particularly that of HPI hydroperoxidase, in other *rcsC* mutants may help resolve this issue.

The *hps-21*::*lacZ* fusion mutant exhibited dual regulation by RpoS and OxyR. The fusion was mapped to an ORF, *f497* (8, 38), whose product is similar to arylsulfatase enzymes (Table 8). Human intestinal micro flora uses arylsulfatase enzymes to break down steroid sulfates in the bile acid and subsequently uses the sulfur for dissimilation (48), while the steroid backbone is reabsorbed by the intestine (18). Often intestinal microflora uses chondroitin sulfate, a mucopolysaccharide of the intestinal tissue, as the sole carbohydrate source (34). The breakdown of chondroitin sulfate to unsulfated di- and monosaccharides is catalyzed by bacterial sulfatases (34). Since the functions of these sulfatases are required under conditions of carbon starvation, it is not surprising that their expression is σ^S dependent (Fig. 3), the sigma factor that directs RNA polymerase to promoters of genes required for survival during starvation. The genes near the bacterial origin of replication are highly conserved, and their functions may play an important role during cell growth (31); however, the independent regulation of a putative metabolic gene by OxyR is unclear at this time, and further studies are required.

One of the *hps* genes showing twofold hydrogen peroxide inducibility and OxyR dependence (complementation with plasmid-borne *oxyR*), *hps-4* (Table 8), was identified as the *hemF* gene, encoding an aerobically induced enzyme, coproporphyrinogen III oxidase, in protoheme IX biosynthesis (45). Protoheme IX is required for activity of both HPI(12) and HPII—the latter as heme d, a *cis*-hydroxylated protoheme IX derivative (25). When cultures are exposed to hydrogen peroxide in exponential phase, there is a rapid increase in *katG* (structural gene for HPI hydroperoxidase) transcription (28, 39). However, to form a functional HPI hydroperoxidase to deal with the challenge, the cell needs to synthesize an adequate amount of protoheme IX. A deficiency is expected to render the cell vulnerable to hydrogen peroxide challenge, as observed for the *hps-4* mutant (Fig. 1). Early-stationary-phase cultures of the *hps-4*::*lacZ* mutant strain had significantly lower levels of both HPI and HPII catalase expression compared to those of an isogenic wild-type strain, as determined by catalase zymograms (35a). The residual catalase activity in this mutant strain is probably due to the synthesis of protoheme IX via the alternate biosynthetic pathway. Since the *hemF* gene product is required for functional HPI catalase synthesis, it is not surprising that *hemF* and *katG* are regulated by OxyR. Thus, *hemF* is also a member of the OxyR regulon.

We presume that the *lacZ* transcription in fusions that are opposite in orientation to the known promoters are probably driven by promoterlike elements in the 3′ regions of the known genes. Although the ORF causing the Hps phenotype in *hps-5* (*thiG*) is yet to be determined (5), it is possible that this fusion is driven by the promoter of *htrC*, a heat shock-inducible gene (32). Our speculation is based on the fact that five proteins, which are members of the hydrogen peroxide regulon, show elevated levels of expression under conditions of heat shock (28). Of these five overexpressing proteins (F52a, E89, D64a, C69, and E79), three are regulated by OxyR (F52a, E89, and D64a) and the identities of only two members are known (F52a, a component of alkyl hydroperoxidase reductase; and C69, DnaK protein). Alkyl hydroperoxidase breaks down hydrogen peroxide, while DnaK may be involved in the repair of peroxidatively damaged DNA. It may also be speculated that DnaK protein, in its capacity as a molecular chaperone, is involved in proper protein folding under oxidizing conditions within the cell.

It is not clear which promoter is involved in the expression of the *lacZ* fusion in *hps-24*::*lacZ* (noncoding strand of *uvrD*); the probable promoter(s) driving the expression may be that of either ORF *f125* or *f161* (13). It is also possible that the insertions in the noncoding strands of known genes cause a polar effect, thereby preventing the expression of essential genes located on the 3′ end and resulting in the hydrogen peroxide-sensitive phenotype.

Our screen failed to identify any previously known OxyR-regulated genes. Since we screened for lac^+ fusion mutants that were hydrogen peroxide sensitive, our screen would not detect any insertion opposite in orientation to an OxyR-regulated promoter. Thus, a hydrogen peroxide-sensitive phenotype caused by an insertional mutation in opposite orientation to any known OxyR-regulated genes would not be detected. In the largely uncharacterized OxyR-independent fusion mutants (class I), some of the insertions may have taken place in known OxyR-regulated genes but in opposite orientation to the OxyR-regulated promoter, resulting in a hydrogen peroxide phenotype where the expression of the *lacZ* gene is directed by an unrelated OxyR-independent promoter element. These questions can be addressed with further characterization of class I fusion mutants.

Class III fusion mutants showed increased *lacZ* expression in an Δ*oxyR* background. The expression pattern of the *hps-19*::*lacZ* mutant suggests that the promoter element(s) is recognized by both RpoS and OxyR and that OxyR acts as a repressor, as is evident from its own expression (10, 44). The class III fusions indicate that OxyR acts as a repressor and represses a group of genes in *E. coli.*

We have attempted to identify the members of the regulon that are induced when *E. coli* is subjected to oxidative stress generated by exposure to hydrogen peroxide and that have protective functions within the cell. Our approach has identified not only additional members of the OxyR-activated regulon but also members of an as-yet-unknown OxyR-repressed regulon.

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