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RpoS- and OxyR-Independent Induction of HPI Catalase at Stationary Phase in *Escherichia coli* and Identification of *rpoS* Mutations in Common Laboratory Strains

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A rapid spectrophotometric assay to determine the activities of HPI and HPII catalases in *Escherichia coli* extracts has been developed. This assay is based upon the differential heat stabilities of the two enzymes and offers significant advantages over previous methods for quantitation of their activities. Measurement of catalase activities in extracts of various mutant strains confirmed the ability of this method to accurately distinguish the two activities. Contrary to previously published results, HPI catalase activity was observed to increase at stationary phase in strains lacking the stationary-phase sigma factor σ^s (RpoS). This increase was independent of OxyR and also occurred in a strain lacking the HPII structural gene, *katE*. These results suggest a potential novel pathway for HPI induction in response to increased oxidative stress in the absence of HPII. Measurement of HPII activity in strains carrying mutations in *pcm* (encoding the L-isoaspartyl protein methyltransferase) and *surE* led to the finding that these strains also have an amber mutation in *rpoS*; sequencing demonstrated the presence of this mutation in several commonly used laboratory strains of *E. coli*, including AB1157, W1485, and JC7623.

Escherichia coli has two catalase enzymes, HPI and HPII, which catalyze the dismutation of hydrogen peroxide to water and oxygen (24). HPI, the product of the *katG* gene, is transcriptionally induced during logarithmic growth in response to low concentrations of hydrogen peroxide. This induction requires the positive activator OxyR, which directly senses oxidative stress and is activated by conformational change upon oxidation occurring at a key cysteine residue (37). HPII, on the other hand, is not peroxide inducible; its gene, *katE*, is transcribed at the transition from exponential growth to stationary phase by RNA polymerase containing the alternative sigma subunit σ^s, the product of the *rpoS* (or *katF*) gene and a key player in survival of stationary phase and other stresses (25, 30).

Interest has been focused on these two catalases not only because of their important roles in protecting cells against the effects of oxidative stress (6) but also because of their suitability as reporter enzymes in the study of global regulatory systems. Quantitation of HPII activity provides a convenient means of monitoring the level of σ^s (45). Similarly, HPI can serve as a measure of the activation of the OxyR stress response regulon by peroxide or other agents (12).

Several biochemical assays for catalase activity have been developed in order to facilitate the study of these enzymes and their regulatory systems. Qualitatively, catalase can be demonstrated by simply dropping peroxide on a bacterial colony and observing bubbles arising from the evolution of oxygen (15). Quantitation of catalase activity can be accomplished either by using a spectrophotometer to measure reduction in absorbance at 240 nm as hydrogen peroxide (H_2O_2) is consumed (2) or by measurement of oxygen evolution with an oxygen electrode (33). However, neither of these methods is entirely satisfactory for the differential quantitation of the two catalase activities in *E. coli*, the major drawback being that the activities of HPI and HPII cannot be distinguished. In order to make differential measurements, some authors have used one of these methods to determine total catalase levels separately for *katE* and *katG* mutants (9, 24). Alternatively, the two catalases can be clearly distinguished by activity staining after nondenaturing gel electrophoresis (5, 10). In this case, however, quantitation relies on densitometry, which gives only a relative measurement and may be compromised by nonlinearity.

In the present paper, we describe a new assay for catalase activity in *E. coli* extracts which combines the ease and rapidity of quantitative measurement by the spectrophotometric method with the ability to distinguish the two catalase activities. By applying this method to several strains carrying mutations in the catalase genes or their regulatory factors, we have demonstrated its ability to accurately discriminate between HPI and HPII. In agreement with published results (24), we observed a modest induction of HPI at stationary phase; contrary to previous findings, however, we found this increased HPI activity to be independent of RpoS. Furthermore, we have shown that the induction does not involve OxyR and also occurs in a *katE* mutant, suggesting that it may represent a previously undiscovered mechanism for cellular response to increased oxidative stress.

We have also applied our assay to the indirect measurement of RpoS activity in strains carrying mutations in *pcm* and *surE*, which form an operon located about 1.2 kb upstream of *rpoS* (22). The *pcm* gene encodes a protein methyltransferase which is specific for L-isoaspartyl residues (7) and which is believed to function in the repair of spontaneous protein damage (42). The biochemical function of *surE* is unknown, but mutations in either gene result in some of the same phenotypes as *rpoS* mutations do (21, 22). The low levels of HPII catalase activity found in these strains led to the discovery that they also have an amber mutation in *rpoS*. The source of this mutation was traced to strain JC7623, a strain which is routinely used in making chromosomal gene replacements, and the same muta-

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TABLE 1. Genotypes of bacterial strains used in this study

Strain	Genotype	Source or reference
MC1000[a]	λ⁻ e14⁻ *araD139* Δ(*araA-leu*)*7697* *galE15 galK16* Δ(*codB-lac*)*3* *rpsL150 mcrB1 relA1 spoT1*	CGSC[b], 3
MC4100	F⁻ *araD139* Δ(*argF-lac*)*U169* *rpsL150 relA1 flbB5301 deoC1 ptsF25 rbsR*	36
CL1010	MC1000 Δ*pcm*::Km[r]	21
JV1012	MC1000 *rpoS13*::Tn*10*	This study
JV1044	MC1000 *katG17*::Tn*10*	This study
JV1045	MC1000 Δ*oxyR*::Km[r]	This study
JV1048	MC1000 Δ*oxyR*::Km[r] *rpoS13*::Tn*10*	This study
JV1065	MC4100 *rpoS13*::Tn*10*	This study
JV1069	MC1000 *katE12*::Tn*10*	This study
UM2	λ⁻ *ara-14 leuB6 azi-6 fhuA23 lacY1 proC83 tsx-67 purE42 glnV44 galK2 trpE38 xthA15 his-208 rfbD1 mgl-51 argG77 rpsL109 glpR201 xylA5 mtl-1 ilvA681 thi-1 metA160 katE32 katG15*	CGSC, 26
MP180	HfrH *thi-1*	P. C. Loewen, 32
UM122	MP180 *rpoS*(*katF*)*13*::Tn*10*	P. C. Loewen, 25
AB1157	F⁻ λ⁻ *thr-1 ara-14 leuB6* Δ(*gpt-proA*)*62 lacY1 tsx-33 glnV44 galK2 qsr' rac hisG4 rfbD1 mgl-51 rpsL31 kdgK51 xylA5 mtl-1 argE3 thi-1*	CGSC, 14
JC7623	AB1157 *recC22 recB21 sbcB15 sbcC201*	CGSC, 13
RM4606	F⁻ λ⁻ *rph-1*[c]	R. Maurer, 40

[a] Used as wild-type parent strain.
[b] *E. coli* Genetic Stock Center, Yale University, New Haven, Conn.
[c] Same as W1485.

tion was subsequently found in other *E. coli* strains in common use.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in this study are listed in Table 1; all are derivatives of *E. coli* K-12. Bacteriophage P1 (36) was used to transduce *katG17*::Tn*10* and Δ*oxyR*::Km[r] markers from strains UM197 (26) and GS09 (41), respectively, into MC1000, generating strains JV1044 and JV1045. Similarly, the *rpoS13*::Tn*10* marker from strain UM122 (25) was transduced into MC1000, JV1045, and MC4100, yielding strains JV1012, JV1048, and JV1065, respectively, and the *katE12*::Tn*10* marker from strain UM120 (25) was transduced into MC1000 to make JV1069.

Preparation of crude extracts and measurement of protein. *E. coli* cells were lysed by sonication of washed cultures which had been resuspended in a phosphate buffer (5 mM potassium phosphate [pH 7.0], 5 mM disodium EDTA, 10% glycerol, 25 μM phenylmethylsulfonyl fluoride) to one-tenth of the original culture volume. Care was taken to avoid destruction of heat-labile catalase activity during sonication; typically, cells were given four rounds of five 0.5-s pulses with a Branson W-350 sonicator and microtip at a power setting of 2 to 3. Tubes were kept on ice between rounds. Debris was pelleted by centrifugation at 4°C for 10 min at 12,000 × *g*. Protein was precipitated from an aliquot of the resulting extract with 10% trichloroacetic acid, and total protein concentration was determined by the method of Lowry et al. (27).

Activity staining for catalase. HPI and HPII catalases present in crude extracts of *E. coli* cells were separated by electrophoresis through a 10% nondenaturing polyacrylamide gel with no stacking gel. Negative staining for catalase activity with horseradish peroxidase and nitroblue tetrazolium was carried out as described previously (5). The two activities were identified by reference to published results from similar experiments (16, 29) and by comparison with an *rpoS* mutant lacking HPII activity.

Quantitation of HPI and HPII activities by spectrophotometry. For total catalase measurement, 5 to 25 μl of the crude extract (usually 2 to 5 mg of total protein/ml) was diluted to 1 ml with distilled water. Assay conditions were essentially the same as those described previously (2). Hydrogen peroxide (0.5 ml of 59 mM H_2O_2 freshly diluted in 50 mM potassium phosphate buffer, pH 7.0) was added, and the absorbance of the samples at 240 nm was measured every 15 s for 1 min. The specific activity of catalase (micromoles of H_2O_2 decomposed per minute per milligram of total protein) was then calculated as follows:

$$\frac{1{,}000 \times \Delta A_{240}/\text{min (average)}}{43.6 \times \text{mg of protein/ml of reaction mixture}}$$

HPII catalase activity (heat stable) was measured by the same procedure, except that an aliquot of the extract was placed in a microcentrifuge tube and heated in a 55°C water bath for 15 min prior to dilution and addition of peroxide. HPI activity (heat labile) was then calculated by subtracting HPII activity (heated extracts) from the total (unheated extracts).

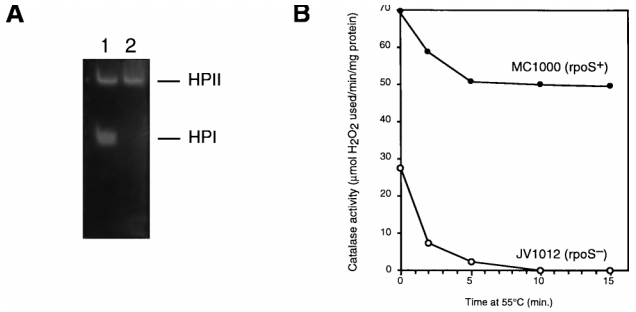

FIG. 1. Loss of HPI catalase activity on heating. (A) Nondenaturing gel showing activity staining of catalase in a crude extract of stationary-phase *E. coli* MC1000. Extracts were either unheated (lane 1) or heated to 55°C for 15 min (lane 2) prior to electrophoresis and staining (see Materials and Methods). The bands representing the HPI and HPII catalases are identified on the right. (B) Spectrophotometric measurement of catalase in extracts of stationary-phase cultures. Total catalase activity was measured as described in the text in extracts of *E. coli* MC1000 (●) and its *rpoS* mutant derivative JV1012 (○) after heating to 55°C for the indicated lengths of time. Points are averages of duplicates.

Detection of RpoS by immunoblotting. For direct measurement of RpoS protein, *E. coli* extracts (typically 20 to 50 μg of total protein) were electrophoresed on 10% polyacrylamide gels and the separated proteins were transferred to nitrocellulose by standard methods (19, 34). Polyclonal antibody against RpoS was obtained from R. Hengge-Aronis (20). RpoS-antibody complexes were visualized with a chemiluminescent detection system (ECL kit; Amersham Corp., Arlington Heights, Ill.).

Oligonucleotides and sequencing. A 1.6-kb fragment of genomic DNA containing the entire *rpoS* gene (including the promoter and the 5′ untranslated region) was amplified by PCR directly from colonies or overnight cultures of appropriate strains (11) with *Taq* polymerase (Promega Corp., Madison, Wis.). Oligonucleotides RPOS1 (5′-GCAGAGCAAGGAGTTGTGAT-3′; bases −650 to −631 of the sense strand relative to the *rpoS* translational start) and RPOS2 (5′-CGGACCTTTTATTGTGCACA-3′; complementary to bases 1040 to 1059) were used as primers for amplification. Single-stranded sequencing of the entire fragment was carried out with these two primers plus two additional ones: RPOS3 (5′-TGATTACCTGAGTGCCTACG-3′; bases −243 to −224) and RPOS4 (5′-TTGGTGAGATTGGTTATTCA-3′; bases 184 to 203). Sequencing was performed with an Applied Biosystems 373A or 377 automated sequencing apparatus. Double-stranded sequencing to confirm the *rpoS*(Am) mutations was done by using primers RPOS3 and RPOS5 (5′-TACCACCAGACGCAAGTTA C-3′; complementary to bases 289 to 308) to amplify and then sequence both strands of a 551-bp DNA fragment including the 5′ end of the *rpoS* gene. Two different PCR products were sequenced for each strain, and strains from two different sources were used in the cases of MC1000 and JC7623.

RESULTS AND DISCUSSION

A rapid method for differential quantitation of HPI and HPII activities. In the course of studies using HPII catalase as a measure of RpoS activity, we observed a significant drop in total catalase activity when preparation of crude extracts included prolonged sonication. Activity staining demonstrated that this decrease was due to the specific inactivation of HPI catalase activity. As shown in Fig. 1A, a briefly sonicated extract of stationary-phase *E. coli* MC1000 (the parental strain for these experiments) had both HPI and HPII activities, but HPI appeared to be very sensitive to heating, and its activity was selectively abolished when the extract was incubated for 15 min at 55°C. Using a quantitative spectrophotometric assay (2), we found that all catalase activity in extracts of strain

TABLE 2. Catalase activities in catalase structural and regulatory mutants

Strain	Relevant genotype	Catalase activity[a]					
		Exponential phase		Exponential phase, H_2O_2 induced[b]		Stationary phase	
		HPI	HPII	HPI	HPII	HPI	HPII
MC1000	Parent strain	6.8 ± 3.2	6.2 ± 1.3	28.0 ± 0.9	4.0 ± 0.6	13.5 ± 3.2	42.7 ± 5.8
JV1045	*oxyR* mutant	13.0 ± 1.9	8.0 ± 1.9	11.0 ± 3.8	8.0 ± 1.9	17.1 ± 7.9	44.0 ± 11.2
JV1012	*rpoS* mutant	9.4 ± 2.5	1.3 ± 0.2			28.6 ± 5.9	1.2 ± 0.5
JV1044	*katG* mutant					0.0 ± 0.0	42.5 ± 15.2
UM2	*katE katG* double mutant					1.0 ± 0.7	0.5 ± 0.4
MC4100	Parent of JV1065					4.0 ± 1.8	
JV1065	*rpoS* mutant					15.5 ± 0.4	
MP180	Parent of UM122					18.1 ± 3.6	
UM122	*rpoS* mutant					34.3 ± 1.9	

[a] Catalase-specific activities are given in micromoles of H_2O_2 decomposed per minute per milligram of total protein and are averages of at least two trials; the error range represents one standard deviation.

[b] Exponentially growing cells were treated with 60 μM hydrogen peroxide for 30 min prior to harvest.

JV1012, an *rpoS* mutant which should express only HPI catalase, was lost after 15 min at 55°C (Fig. 1B). Less than 10% of the initial activity remained after only 5 min of heating. In MC1000 extracts, however, most of the activity was stable, suggesting that we could exploit the distinct heat stabilities of the two catalases to develop an assay which is rapid and quantitative and distinguishes between HPI and HPII. We thus modified the spectrophotometric method of Beers and Sizer (2), measuring the sum of HPI and HPII activities in one aliquot and HPII activity in a second, heated, aliquot. HPI activity was then obtained from the difference of the two determinations. The heat lability of HPI was previously demonstrated by Meir and Yagil (28), and our results are consistent with their original findings.

HPI and HPII catalase activities in catalase structural and regulatory mutants. The major potential benefit of our catalase assay method is the differential quantitation of HPI and HPII catalase activities. To test the validity of our assay, we measured catalase activities for several strains (Table 1) known to carry mutations in the catalase genes or their regulatory factors.

During exponential growth, the activities of both catalases should be low but HPI activity should be inducible by peroxide (35). Table 2 shows the HPI and HPII activities of strains grown to mid-log phase (optical density at 600 nm, ~0.4) and assayed as described above. The parent strain (MC1000) had low levels of both catalases. When 60 μM H_2O_2 was added to the cultures for 30 min prior to making the extracts, however, HPI activity increased fourfold in this strain, consistent with previous results (4). No change in HPII activity was observed. Similar basal levels of catalase activity were observed for an *oxyR* mutant (JV1045), but neither HPI nor HPII was induced by peroxide in this strain: HPI is not inducible in the absence of this positive regulator of *katG* transcription (4). In an *rpoS* mutant (JV1012), which should be unable to transcribe *katE* (24), HPII activity was barely detectable but HPI activity was similar to that in the parent strain.

In cultures grown to stationary phase (measured after 16 to 20 h of growth in Luria-Bertani broth) (Table 2), HPI activity in MC1000 had increased only modestly but HPII activity showed a dramatic induction. These results agree with published reports of a mild increase in *katG* expression as cells approach stationary phase (16, 23, 24) and a major increase in the synthesis of the RpoS-dependent HPII (24). Insertional inactivation of *rpoS* (JV1012) eliminated HPII, but not HPI, activity, while an insertion in the HPI structural gene, *katG* (JV1044), had the opposite effect. Deletion of *oxyR* (JV1045) did not reduce the activity of either catalase at this growth stage.

As expected, mutation of both catalase structural genes (strain UM2) (Table 2) virtually eliminated both HPI and HPII activities. The very low level of activity observed in this strain was at the limit of detection. It is possible that some additional, weak catalase activity is present in the extracts, or there could be a small amount of HPI which is not fully heat inactivated. Alternatively the activity which remains may result simply from leakiness of the mutations, which may be point mutations in UM2. In any case, such a small amount of residual activity should not have a significant effect on the quantitation of HPI and HPII.

In each case, the measured amounts of the heat-labile and heat-stable catalase activities matched those expected for HPI and HPII, respectively. Furthermore, the specific activities determined by our method are in good agreement with values published previously (24). We therefore conclude that HPI and HPII can be accurately distinguished and quantitated by this simple, rapid assay without the need for gels or densitometry; this method also appears to be suitable for monitoring the activities of regulatory factors such as σ^s and OxyR.

Effect of *rpoS* mutations on HPI accumulation at stationary phase. Ivanova et al. (16) reported an increase in HPI catalase activity at stationary phase, which they attributed to RpoS-dependent transcription of *katG*. In *rpoS* mutants, these authors detected little or no activity by activity staining. While we observed a similar rise in HPI levels (compare exponential with stationary-phase levels for MC1000 in Table 2), we were surprised to find that inactivation of *rpoS* did not prevent the increase. To the contrary, in fact, the *rpoS* mutant (JV1012) exhibited about 50% higher HPI activity than the parent strain. This result was consistently obtained in assays of many different extracts, and the presence of significant levels of HPI was further confirmed by means of activity gels (data not shown).

We investigated the possibility that differences in genetic background accounted for these unexpected results by measuring HPI activity in strain MC4100 (used by Ivanova et al. as the parent strain for most experiments) and the isogenic *rpoS* mutant JV1065 as well as in the original *rpoS13*::Tn*10* strain, UM122 (25), and its parent, MP180 (also tested by Ivanova et al.). In our hands, both these pairs of strains showed the same

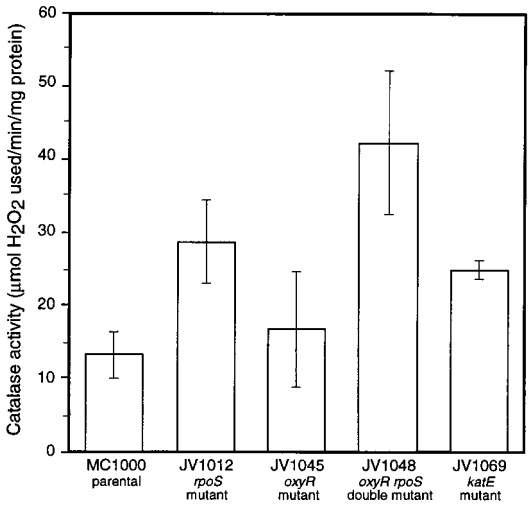

FIG. 2. Effect of *oxyR* and *katE* mutations on HPI catalase activity. Catalase activity in extracts of *E. coli* cultures grown to stationary phase was measured by the spectrophotometric method described in Materials and Methods; only HPI (heat labile) activity is shown. Bars represent averages of at least two trials; error bars show one standard deviation. Strain JV1045 is a Δ*oxyR*::Km^r derivative of MC1000, and JV1048 is JV1045 carrying *rpoS13*::Tn*10*. Strain JV1069 is MC1000 carrying a *katE12*::Tn*10* mutation.

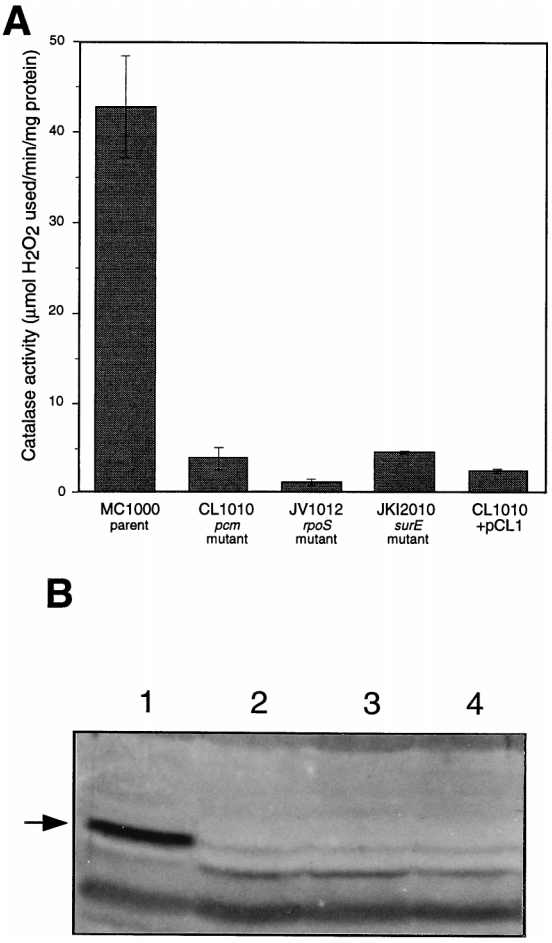

FIG. 3. HPII catalase activity demonstrates reduced RpoS in *pcm* and *surE* mutant strains. (A) HPII catalase activity in *pcm* and *surE* mutants. Catalase activity was measured as before; only HPII (heat stable) activity is shown. Bars represent averages of at least three trials; error bars show one standard deviation. Strain CL1010 has a Δ*pcm*::Km mutation, JKI2010 has a *surE*::Km insertion, and plasmid pCL1 carries wild-type copies of both *pcm* and *surE*. (B) Confirmation of reduced RpoS by immunoblotting. Anti-RpoS antibody was used to detect RpoS (arrow) among proteins from crude extracts separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose as described in Materials and Methods. Lanes: 1, MC1000 (wild type [wt]; 2, CL1010 (*pcm* mutant); 3, JV1012 (*rpoS* mutant); 4, JKI2010 (*surE* mutant).

pattern as we observed for MC1000: HPI activity at stationary phase increased when *rpoS* was mutated (Table 2).

We are uncertain how to explain the apparent contradiction between our results and those of Ivanova et al. (16). We have observed that HPI is slightly more heat sensitive at stationary phase in an *rpoS* mutant strain than in MC1000 ($t_{1/2}$ for inactivation at 55°C averaged 2.5 min for *rpoS*$^+$ versus 1.5 min for the *rpoS* mutant); while Ivanova et al. do not describe their method of making cell extracts in detail, it is possible that their preparations were heated (e.g., by sonication) so that HPI activity was selectively lost in the *rpoS* mutant strains. However, these authors also present evidence for diminished *katG* transcription at stationary phase in *rpoS* mutants, and corroborating results have been reported (unpublished results from H. Schellhorn cited in reference 23). It is not clear at present how such a transcriptional dependence on *rpoS* might be reconciled with our results.

Evidence for a novel pathway of HPI induction in response to oxidative stress. One hypothesis to explain the activation of HPI at stationary phase in *rpoS* mutants would be that the cells are experiencing increased oxidative stress due to the absence of HPII catalase. Since OxyR is a known activator of *katG* transcription and is itself sensitive to oxidation (37), it might become oxidized in *rpoS* mutant strains even in the absence of exogenous H_2O_2 and thus induce HPI synthesis. Indeed, H_2O_2 has been shown to accumulate to significant levels during normal growth of *E. coli* in culture (8, 9).

To test this hypothesis, we measured HPI levels in an *oxyR* mutant (JV1045) and in an *oxyR rpoS* double mutant (JV1048) at stationary phase. As shown in Fig. 2, deletion of *oxyR* alone had little effect, consistent with the published finding that OxyR is not involved in the stationary-phase expression of HPI (16). However, we again observed an increase in HPI in the *oxyR rpoS* double mutant strain, demonstrating that this HPI induction is both *oxyR* and *rpoS* independent. Furthermore, mutation of both *oxyR* and *rpoS* repeatably resulted in somewhat more HPI production than did mutation of *rpoS* alone.

Clearly, oxidation of *oxyR* is not required for the observed stationary-phase induction of HPI. Nonetheless, the induction could still be a response to increased oxidative stress, albeit via a pathway which has not been reported previously. To examine this possibility, we measured HPI levels in a strain in which the HPII structural gene, *katE*, had been insertionally inactivated (Fig. 2, strain JV1069). This strain also showed an induction of HPI at stationary phase relative to that of the parent strain, MC1000, even though *rpoS* is intact. This supports the hypothesis that HPI activity is increasing in response to elevated oxidative stress in the HPII-deficient cells; we conclude from the *oxyR* and *rpoS* mutants that *E. coli* must have an additional mechanism by which it can sense its oxidative state and induce HPI activity.

In summary, the data presented here suggest the existence of a secondary pathway for HPI induction at stationary phase in *E. coli*. Because HPI levels are elevated in *katE* mutants as well as *rpoS* mutants, we propose that this may be a response to increased oxidative stress in the absence of HPII catalase. We

have demonstrated that this is not an OxyR-regulated event, and the transcription results discussed above may suggest some form of posttranscriptional control. Further investigation of this apparently novel RpoS- and OxyR-independent pathway of HPI induction may yield new members of the *E. coli* oxidative stress response regulatory network.

Deficiency of RpoS in *pcm* and *surE* mutant strains due to amber mutations. We have previously reported the construction and characterization of strains mutated in two genes in an operon just upstream of *rpoS*: *pcm*, encoding the L-isoaspartyl protein carboxyl methyltransferase, believed to function in protein repair, and *surE*, whose function is not yet known (21, 22). Mutations in either of these genes appeared to have phenotypes similar to but less severe than those of *rpoS* mutants, including reduced survival in stationary phase or after exposure to thermal, oxidative, or osmotic stresses. In order to determine whether these upstream mutations were affecting RpoS levels, we measured HPII catalase activity as a reporter for RpoS and also measured RpoS protein directly by immunoblotting. Measurement of HPII catalase activity (Fig. 3A) suggested that the *pcm* (CL1010) and *surE* (JKI2010) mutants had only 5 to 10% of normal (MC1000) RpoS activity, though significantly more than an *rpoS*::Tn*10* strain (JV1012). A plasmid (CL1010 + pCL1) which carries both *surE* and *pcm* and overexpresses *pcm* by about 50-fold (data not shown) failed to restore HPII activity. No RpoS protein could be detected by immunoblotting in any of the three mutants (Fig. 3B, lanes 2 to 4), although the relatively high background observed with this antibody could have masked small amounts of RpoS.

Since this RpoS defect was not complemented by wild-type *pcm* or *surE* and the mutations did not appear to affect the transcription of *rpoS* (data not shown), we sequenced the chromosomal copy of *rpoS* to determine whether we might have inadvertently introduced or selected for secondary mutations. In MC1000, the *rpoS* sequence was identical to that generated by the *E. coli* genome sequencing project (GenBank accession no. AE000358). The *pcm* and *surE* mutant strains (CL1010 and JKI2010, respectively), however, had a single-base change relative to this sequence, a C-to-T change at codon 33 of *rpoS*. This mutation changes a CAG codon, specifying glutamine, to a TAG amber nonsense codon.

Since MC1000 carries no known suppressor mutations, this premature stop signal near the 5′ end of the gene presumably explains the greatly reduced levels of *rpoS* observed in these strains. The *rpoS* mutation could account for some or all of the phenotypes which we have observed so far for the *pcm* and *surE* mutants, and reevaluation of the effects of these mutations will be necessary. The synthesis of HPII at 5 to 10% of wild-type levels is probably due to leakiness (readthrough) of the nonsense codon, which may not be in as favorable a context as a natural stop codon (31). The amber mutation has been designated *rpoS396* by the *E. coli* Genetic Stock Center.

Additional sequencing revealed the probable source of this mutation: the amber mutation was also found in strain JC7623. This strain carries *recBC* and *sbcBC* mutations (Table 1) and is commonly used for introducing mutations into the *E. coli* chromosome (43). The *pcm* and *surE* chromosomal mutations were originally constructed in this strain and transduced to MC1000 with bacteriophage P1; these genes are tightly linked to *rpoS*, and the two mutations were undoubtedly cotransduced. We also found the same mutation in strain AB1157, a parent of JC7623 used by many researchers as a wild-type parental strain, and in RM4606 (identical to W1485), which is very close to wild-type *E. coli* K-12. Thus, this mutation may be quite common in laboratory strains of *E. coli.*

It has become apparent from the work of several researchers that variations occur in *rpoS* at a relatively high frequency. Various *rpoS* mutations have been found in apparently wild-type strains or in strains which have been subjected to long-term starvation (17, 18, 38, 39, 44, 45), including other amber mutations which affect the C-terminal extension of *rpoS* but do not completely inactivate it. The amber mutation described here has so far been reported only in a strain with a known RpoS$^-$ phenotype (17).

The presence of an N-terminal amber mutation in commonly used strains suggests a need for caution in the study of RpoS and RpoS-dependent genes in these strains. Although AB1157 and its descendants carry the *glnV44* (*supE44*) amber suppressor allele, which probably mitigates the effects of the *rpoS*(Am) mutation, RpoS levels are nonetheless likely to be lower than would be expected in a truly wild-type strain. For example, although the two strains are not directly comparable, we observed that HPII catalase activity in stationary-phase JC7623 was only about 35% of that in MC1000 (data not shown).

Since not all *E. coli* K-12 strains carry this mutation, it must either have arisen independently in more than one lineage or have been reverted in some lineages. It is possible that this site is a hot spot for mutation in *rpoS*: the C-to-T change occurs in the sequence ACAGG, which matches four out of five bases of the *dcm*-encoded DNA cytosine methyltransferase methylation consensus sequence CCAGG. It has recently been reported that such near-consensus sites can be methylated, at least when this methyltransferase is overproduced, resulting in an increased frequency of C-to-T transitions at these sites (1). It is also interesting to note the recent finding that at least some isolates of W3110, a derivative of W1485, have a TAT (tyrosine) codon at this site (18), suggesting that the amber mutation (TAG) may have arisen first and then been pseudoreverted by a second mutation to restore a sense codon. The occurrence of this second mutation in this nonsuppressing lineage—contrasted with the maintenance of the amber mutation in the amber-suppressing AB1157 lineage—implies significant selective pressure to maintain a functional copy of *rpoS.*

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ADDENDUM IN PROOF

After this report was submitted for publication, E. Glater and A. C. Vollmer presented corroborating results on HPI regulation (Abstracts of the 97th General Meeting of the American Society for Microbiology 1997, abstr. I-7, p. 323, 1997). These researchers also observed an increase in *katG* expression at stationary phase in *rpoS* mutants. Their assay utilized a transcriptional fusion of the *katG* promoter to the *luxCDABE* operon, suggesting that *rpoS*-independent stationary-phase induction of *katG* may be regulated at the transcriptional level.

REFERENCES

1. **Bandaru, B., J. Gopal, and A. S. Bhagwat.** 1996. Overproduction of DNA cytosine methyltransferase causes methylation and C→T mutations at non-canonical sites. J. Biol. Chem. **271:**7851–7859.
2. **Beers, R. F., Jr., and I. W. Sizer.** 1951. A spectrophotometric method for

measuring the breakdown of hydrogen peroxide by catalase. J. Biol. Chem. **196:**133–140.
3. **Casadaban, M. J., and S. N. Cohen.** 1980. Analysis of gene control signals by DNA fusion and cloning in *Escherichia coli*. J. Mol. Biol. **138:**179–207.
4. **Christman, M. F., R. W. Morgan, F. S. Jacobson, and B. N. Ames.** 1985. Positive control of a regulon for defenses against oxidative stress and some heat-shock proteins in *Salmonella typhimurium*. Cell **41:**753–762.
5. **Clare, D. A., M. N. Duong, D. Darr, F. Archibald, and I. Fridovich.** 1984. Effects of molecular oxygen on detection of superoxide radical with nitroblue tetrazolium and on activity stains for catalase. Anal. Biochem. **140:**532–537.
6. **Farr, S. B., and T. Kogoma.** 1991. Oxidative stress responses in *Escherichia coli* and *Salmonella typhimurium*. Microbiol. Rev. **55:**561–585.
7. **Fu, J. C., L. Ding, and S. Clarke.** 1991. Purification, gene cloning, and sequence analysis of an L-isoaspartyl protein carboxyl methyltransferase from *Escherichia coli*. J. Biol. Chem. **266:**14562–14572.
8. **González-Flecha, B., and B. Demple.** 1995. Metabolic sources of hydrogen peroxide in aerobically growing *Escherichia coli*. J. Biol. Chem. **270:**13681–13687.
9. **González-Flecha, B., and B. Demple.** 1997. Homeostatic regulation of intracellular hydrogen peroxide concentration in aerobically growing *Escherichia coli*. J. Bacteriol. **179:**382–388.
10. **Gregory, E. M., and I. Fridovich.** 1974. Visualization of catalase on acrylamide gels. Anal. Biochem. **58:**57–62.
11. **Gussow, D., and T. Clackson.** 1989. Direct clone characterization from plaques and colonies by the polymerase chain reaction. Nucleic Acids Res. **17:**4000.
12. **Hausladen, A., C. T. Privalle, T. Keng, J. DeAngelo, and J. S. Stamler.** 1996. Nitrosative stress: activation of the transcription factor OxyR. Cell **86:**719–729.
13. **Horii, Z. I., and A. J. Clark.** 1973. Genetic analysis of the RecF pathway to genetic recombination in *Escherichia coli* K-12: isolation and characterization of mutants. J. Mol. Biol. **80:**327–344.
14. **Howard-Flanders, P., E. Simson, and L. Theriot.** 1964. A locus that controls filament formation and sensitivity to radiation in *Escherichia coli* K-12. Genetics **49:**237–246.
15. **Huisman, G., and R. Kolter.** 1994. Regulation of gene expression at the onset of stationary phase in *Escherichia coli*, p. 21–40. *In* P. Piggot, C. P. Moran, Jr., and P. Youngman (ed.), Regulation of bacterial differentiation. American Society for Microbiology, Washington, D.C.
16. **Ivanova, A., C. Miller, G. Glinsky, and A. Eisenstark.** 1994. Role of *rpoS* (*katF*) in *oxyR*-independent regulation of hydroperoxidase I in *Escherichia coli*. Mol. Microbiol. **12:**571–578.
17. **Ivanova, A., M. Renshaw, R. V. Guntaka, and A. Eisenstark.** 1992. DNA base sequence variability in *katF* (putative sigma factor) gene of *Escherichia coli*. Nucleic Acids Res. **20:**5479–5480.
18. **Jishage, M., and A. Ishihama.** 1997. Variation in RNA polymerase sigma subunit composition within different stocks of *Escherichia coli* W3110. J. Bacteriol. **179:**959–963.
19. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:**680–685.
20. **Lange, R., and R. Hengge-Aronis.** 1994. The cellular concentration of the σ^s subunit of RNA polymerase in *Escherichia coli* is controlled at the levels of transcription, translation, and protein stability. Genes Dev. **8:**1600–1612.
21. **Li, C., and S. Clarke.** 1992. A protein methyltransferase specific for altered aspartyl residues is important in *Escherichia coli* stationary-phase survival and heat-shock resistance. Proc. Natl. Acad. Sci. USA **89:**9885–9889.
22. **Li, C., J. K. Ichikawa, J. J. Ravetto, H.-C. Kuo, J. C. Fu, and S. Clarke.** 1994. A new gene involved in stationary-phase survival located at 59 minutes on the *Escherichia coli* chromosome. J. Bacteriol. **176:**6015–6022.
23. **Loewen, P. C., and R. Hengge-Aronis.** 1994. The role of the sigma factor σ^s (KatF) in bacterial global regulation. Annu. Rev. Microbiol. **48:**53–80.
24. **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.
25. **Loewen, P. C., and B. L. Triggs.** 1984. Genetic mapping of *katF*, a locus that with *katE* affects the synthesis of a second catalase species in *Escherichia coli*. J. Bacteriol. **160:**668–675.
26. **Loewen, P. C., B. L. Triggs, C. S. George, and B. E. Hrabauchuk.** 1985. Genetic mapping of *katG*, a locus that affects synthesis of the bifunctional catalase-peroxidase hydroperoxidase I in *Escherichia coli*. J. Bacteriol. **162:**661–667.
27. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:**265–275.
28. **Meir, E., and E. Yagil.** 1985. Further characterization of the two catalases in *Escherichia coli*. Curr. Microbiol. **12:**315–320.
29. **Mulvey, M. R., P. A. Sorby, B. L. Triggs-Raine, and P. C. Loewen.** 1988. Cloning and physical characterization of *katE* and *katF* required for catalase HPII expression in *Escherichia coli*. Gene **73:**337–345.
30. **Mulvey, M. R., J. Switala, A. Borys, and P. C. Loewen.** 1990. Regulation of transcription of *katE* and *katF* in *Escherichia coli*. J. Bacteriol. **172:**6713–6720.
31. **Parker, J.** 1989. Errors and alternatives in reading the universal genetic code. Microbiol. Rev. **53:**273–298.
32. **Pearson, M. L.** 1972. The role of adenosine-3′-5′-cyclic monophosphate in the growth of bacteriophage lambda. Virology **49:**605–609.
33. **Rorth, M., and P. K. Jensen.** 1967. Determination of catalase activity by means of the Clark oxygen electrode. Biochim. Biophys. Acta **139:**171–173.
34. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Press, Cold Spring Harbor, N.Y.
35. **Schellhorn, H. E.** 1995. Regulation of hydroperoxidase (catalase) expression in *Escherichia coli*. FEMS Microbiol. Lett. **131:**113–119.
36. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
37. **Storz, G., L. A. Tartaglia, and B. N. Ames.** 1990. Transcriptional regulator of oxidative stress-inducible genes: direct activation by oxidation. Science **248:**189–194.
38. **Strom, A. R., and I. Kaasen.** 1993. Trehalose metabolism in *Escherichia coli*: stress protection and stress regulation of gene expression. Mol. Microbiol. **8:**205–210.
39. **Styrvold, O. B., and A. R. Strøm.** 1991. Synthesis, accumulation, and excretion of trehalose in osmotically stressed *Escherichia coli* K-12 strains: influence of amber suppressors and function of the periplasmic trehalase. J. Bacteriol. **173:**1187–1192.
40. **Taylor, M., and C. Yanofsky.** 1964. Transformation of bacterial markers and transfer of phage markers with DNA isolated from λ-Φ-80 hybrid phage carrying the tryptophan genes of *E. coli*. Biochem. Biophys. Res. Commun. **17:**798–804.
41. **Toledano, M. B., I. Kullik, F. Trinh, P. T. Baird, T. D. Schneider, and G. Storz.** 1994. Redox-dependent shift of OxyR-DNA contacts along an extended DNA-binding site: a mechanism for differential promoter selection. Cell **78:**897–909.
42. **Visick, J. E., and S. Clarke.** 1995. Repair, refold, recycle: how bacteria can deal with spontaneous and environmental damage to proteins. Mol. Microbiol. **16:**835–845.
43. **Winans, S. C., S. J. Elledge, J. H. Kreuger, and G. C. Walker.** 1985. Site-directed insertion and deletion mutagenesis with cloned fragments in *Escherichia coli*. J. Bacteriol. **161:**1219–1221.
44. **Zambrano, M. M., and R. Kolter.** 1996. GASPing for life in stationary phase. Cell **86:**181–184.
45. **Zambrano, M. M., D. A. Siegele, M. Almirón, A. Tormo, and R. Kolter.** 1993. Microbial competition: *Escherichia coli* mutants that take over stationary phase cultures. Science **259:**1757–1760.