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Transcriptional Regulation of the *Escherichia coli oxyR* Gene as a Function of Cell Growth

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The *oxyR* regulon plays a central role in the defense of *Escherichia coli* against the endogenous oxidative damage associated with active aerobic growth. Here we have studied the transcriptional regulation of *oxyR* in *E. coli* growing aerobically in rich medium. Expression of a single-copy *oxyR′::lacZ* reporter construct varied sixfold along the growth curve, with the highest value at 4 to 6 h of growth (~14×10^8 cells · ml^{-1}). Direct measurements of *oxyR* mRNA by primer extension showed the same biphasic expression but with a peak somewhat earlier in cell growth (2 to 3 h; ~3.5×10^8 cells · ml^{-1}). The results of immunoblotting experiments demonstrated that the level of OxyR protein exhibits the same biphasic expression. Mutant strains lacking adenylate cyclase (*cya*) or Crp protein (*crp*) failed to increase *oxyR* expression during exponential growth. On the other hand, an *rpoS* mutation allowed *oxyR* expression to continue increasing as the cells entered stationary phase. Consistent with a biological role for increased levels of OxyR during exponential growth, the *crp cya* strain had lower activities of catalase hydroperoxidase I and glutathione reductase and an increased sensitivity to exogenously added hydrogen peroxide. These results suggest that the Crp-dependent upregulation of *oxyR* in exponential phase is a component of a multistep strategy to counteract endogenous oxidative stress in actively growing *E. coli* cells.

The adaptive responses to oxidative stress in *Escherichia coli* include two regulons controlled by the *oxyR* and *soxRS* genes, which orchestrate defense gene induction triggered by hydrogen peroxide or superoxide-generating systems, respectively (15). Exposing wild-type *E. coli* to micromolar levels of H_2O_2 induces a protective response that confers resistance to subsequent exposure to millimolar H_2O_2 concentrations (6, 8). About 8 of the 30 to 40 proteins induced after treatment with H_2O_2 are products of the *oxyR* regulon genes (6, 13, 25).

To date, the possible transcriptional regulation of the *oxyR* gene has not been extensively studied, except to note that the level of *oxyR* mRNA was not increased after H_2O_2 activation of the *oxyR* regulon (7). Although overproduction of OxyR from multicopy plasmids was not followed by superinduction of the OxyR-dependent enzymes upon H_2O_2 treatment (30), several lines of evidence suggest that regulation of *oxyR* expression has an important biological role. For example, the glucose repression described for catalase (13, 25) may not arise from direct regulation of *katG* by Crp, since no functional Crp-binding site has been found in the *katG* promoter. Also, the OxyR-mediated response to equivalent H_2O_2/catalase ratios varies along the growth curve, being maximal during exponential phase and almost negligible during early stationary phase (11). Finally, the close correlation between the rates of production of O_2^- and H_2O_2 and the respiratory activity of bacterial cells (11), together with the role for OxyR in the homeostatic regulation of the intracellular concentration of H_2O_2 (12), points to a regulation of OxyR activity by oxygen tension or respiration.

In this study, we monitored the variation of *oxyR* expression in aerobically growing bacteria and evaluated possible regulators of the *oxyR* gene. We also measured the level of OxyR-dependent activities and the sensitivity to H_2O_2 in strains with decreased expression of *oxyR*.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Table 1 lists the *E. coli* strains and plasmids used in this study. The bacteriophage λRS45 (*bla′-′lacYA*$^+$) (29) was used to insert the *oxyR′::lacZ* fusion into the chromosomal DNA by recombination in strain MC4100 carrying plasmid pAQ23, using methods previously described (29). Plasmid pAQ23 contains the 5′ 15% of *oxyR* cloned into pRS415 (7). The resulting lysogens, BGF930, BGF932, and BGF940, were identified by their Lac$^+$ Amp^s phenotype. Attempts to introduce the single-copy fusion into strains CA8000, CA8306, and CA8445 were unsuccessful. Strain BGF612 was constructed by cotransduction (24) of Δ*cya* (50% linked to Km^r) into strain BGF930. Strains BGF950, BGF960, and BGF970 were constructed by cotransduction into strain MC4100 of Tn*10* markers linked to the *fnr-250*, *arcB1*, and *arcA2* alleles in strains RK5288, ECL594, and ECL906, respectively. Strain BGF1030 was constructed by transduction of *katF1*::*kan* from strain ZK1001 into BGF930.

Cells were inoculated into LB broth (24) containing the appropriate antibiotics and incubated overnight at 37°C with gentle shaking (100 rpm). For experiments, the cultures grown overnight were diluted 100-fold into fresh LB broth and incubated at 37°C for the times indicated in the figures. Antibiotics were used at the following concentrations (in micrograms per milliliter): ampicillin, 100; tetracycline, 12.5; streptomycin, 50; and kanamycin, 50.

Anaerobic cell growth was performed in 2-ml vials fitted with butyl rubber stoppers as previously described by Jones and Gunsalus (20).

β-Galactosidase. β-Galactosidase activity in sodium dodecyl sulfate (SDS)-CH_3Cl-treated cells was determined by the method of Miller (24) and normalized to the cell density. Absorbance and optical density measurements were carried out in a Perkin-Elmer model Lambda 3A spectrophotometer (Perkin-Elmer, Oak Brook, Ill.).

RNA isolation and primer extension. Samples containing ~10^{10} cells (5 to 20 ml) were taken at the indicated times of growth and placed on ice, and total RNA was extracted by a modified version of the hot-phenol extraction method of Emory and Belasco (9). Briefly, the chilled samples were centrifuged and the cells were resuspended in 125 μl of ice-cold 0.3 M sucrose–0.01 M sodium acetate (pH 4.5). After addition of 125 μl of 2% SDS–0.01 M sodium acetate (pH 4.5), the cell suspension was heated for 3 min at 70°C and extracted three times for 3 min each at 70°C with 250 μl of hot phenol previously equilibrated with unbuffered water. The RNA was ethanol precipitated and stored at −80°C in 20 mM sodium phosphate (pH 6.5)–1 mM EDTA. The RNA concentration in a sample was determined spectrophotometrically (28). A 20-base oligonucleotide (5′-GGTACTCAAGATCACGAATA-3′) was 5′ end labeled with polynucleotide kinase and [γ-^{32}P]ATP (~8,000 Ci · $mmol^{-1}$). RNA samples (100 μg) were mixed with the labeled primer (15,000 to 30,000 cpm) in water, and the samples

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TABLE 1. Bacterial strains and plasmids used in this study

E. coli strain or plasmid	Relevant genotype or phenotype	Source or reference
Strains		
BGF930	As RK4936, but λ[Φ(*oxyR′*::*lacZ*)]	This work
BGF931	As RK4936, but λ[Φ(*katG′*::*lacZ*)]	11
BGF932	As TA4112, but λ[Φ(*oxyR′*::*lacZ*)]	This work
BGF933	As TA4112, but λ[Φ(*katG′*::*lacZ*)]	11
BGF940	As MC4100, but λ[Φ(*oxyR′*::*lacZ*)]	This work
BGF950	As MC4100, but λ[Φ(*oxyR′*::*lacZ*)] *fnr-250 zcj*::Tn*10*	This work
BGF960	As MC4100, but λ[Φ(*oxyR′*::*lacZ*)] *arcB1 zgl*::Tn*10*	This work
BGF612	As BGF930, but Δ*cya* Km^r	This work
BGF970	As MC4100, but λ[Φ(*oxyR′*::*lacZ*)] *arcA2 zjj*::Tn*10*	This work
BGF1030	As BGF930, but *katF1*::*kan*	This work
CA8000	Hfr H B_1^-	4
CA8306	Hfr H B_1^- Δ*cya*	27
CA8445	Hfr H B_1^- Δ*cya* Δ*crp*	27
ECL594	As ECL547, but *arcB1 zgl*::Tn*10*	16
ECL906	As ECL525, but *arcA2 zjj*::Tn*10*	17
MC4100	Δ(*lac*)*U169 rpsL*	Laboratory stock
M7141	Δ*cya* 50% linked to Km^r	A. Hochschild
RK4936	*araD139* (*argF-lac*)*205 flbB5301 non-9 gyrA219 relA1 rpsL150 metE70 btuB*::Tn*10*	Laboratory stock
RL5288	As MC4100, but *gyrA non* λp1(*209*) Δ(Mu) Φ(*nar-lac*)*218 fnr-250 zcj-637*::Tn*10*	20
RR1	*pro leu rpsL hsdM hsdR endI lacY*	3
TA4112	As RK4936, but Δ(*oxyR-btuB*)*3*	6
ZK1001	As W3110, but Δ*lacU169 tnaZ katF1*::*kan cysC95*::Tn*10*	R. Kolter
Plasmids		
pAT153	Vector Ap^r Tc^r	33
pUC18	Vector Ap^r	35
pUC19	Vector Ap^r	Laboratory stock
pRS415	Vector Ap^r	Laboratory stock
pAQ17	pUC19 containing *oxyR*	7
pAQ23	pRS415 containing *oxyR′*::*lacZ*	7
pAQ24	pRS415 containing *katG′*::*lacZ*	31
pBT22	pAT153 containing *katG*	32
pDEB2	pUC19 containing *rpoS*	2

were heated at 58°C for 5 min and then cooled on ice for 10 min. After this annealing procedure, extension products were generated with the avian myeloblastosis virus reverse transcriptase (Promega, Middleton, Wis.) following the manufacturer's procedure. The products were electrophoresed in denaturing gels containing 8% acrylamide (28).

Immunoblot assay of OxyR. Pellets from 5- to 20-ml cultures (~10^{10} cells) were sonicated in 250 μl of 10 mM Tris buffer (pH 8) containing 20% glycerol, centrifuged at 6,000 × *g* for 15 min, and concentrated 10-fold by evaporation. Samples (~200 μg of total protein) were electrophoresed in an SDS-12% polyacrylamide gel and electroblotted to a nitrocellulose filter. OxyR was detected by using antibodies against an OxyR–β-galactosidase fusion protein (30) and visualized with the enhanced chemiluminescence system (Amersham, Arlington Heights, Ill.).

H_2O_2 concentrations. The intracellular concentration of H_2O_2 was assessed by peroxidase-mediated scopoletin oxidation as previously described (12).

RESULTS

Growth-phase-dependent variation in *oxyR* expression. We monitored *oxyR* expression during growth by introducing a single-copy *oxyR′*::*lacZ* operon fusion into wild-type (BGF930) and Δ*oxyR* (BGF932) strains. β-Galactosidase activity showed a biphasic profile in both strains, with minimal values at ~2 h of outgrowth, and maxima at ~4 h (Fig. 1A). After reaching a peak at mid-exponential phase, *oxyR* expression declined, yielding plateau values of ~200 and ~400 U of β-galactosidase for BGF930 and BGF932, respectively (Fig. 1A).

The *oxyR′*::*lacZ*-directed β-galactosidase activity in the Δ*oxyR* strain (BGF932) was always somewhat higher than that of the wild-type strain (BGF930). This result indicates that the autorepression of *oxyR*, reported by Christman et al. (7) for exponential-phase cultures, does not alter the oscillating pattern of *oxyR* expression during aerobic growth.

We confirmed the results obtained with the reporter fusion by measuring the levels of *oxyR*$^+$ message directly. The steady-state level of *oxyR* mRNA, assessed by primer extension, was 3.5-fold higher during exponential growth (2 to 4 h) than the initial level (Fig. 1B). A similar result was obtained by using Northern blotting to detect *oxyR* mRNA (data not shown). The biphasic profile was similar in shape to that for the *oxyR′*::*lacZ* fusion. However, the induction and maximum for the mRNA level preceded by ~2 h that seen for β-galactosidase expression with the fusion gene (Fig. 1A). This difference is likely due to a delay for protein synthesis and effects of the stability of β-galactosidase.

We checked whether the level of OxyR protein in the bacteria reflected the observed variations in mRNA levels during growth. Polyclonal antibodies against OxyR (30) were employed in immunoblotting experiments with protein samples normalized to 10^9 cells. A typical experiment showed that very little OxyR was present at 1 h of outgrowth and that the protein accumulated steadily through exponential phase and into early stationary phase (Fig. 1C). The OxyR protein level then decreased during stationary phase, with a slight but reproducible increase at 16 h (Fig. 1C). Overall, the pattern of OxyR protein expression paralleled the transcriptional behavior of the *oxyR* gene reasonably well.

Transcriptional regulation of *oxyR*. Since OxyR mediates a response to oxidative stress in *E. coli*, we first evaluated the possibility of oxygen dependence of its growth-phase-dependent regulation. The change in *oxyR* expression during aerobic growth was completely abolished in the absence of oxygen (Fig. 2). Neither addition of exogenous H_2O_2 (7, 30) nor treatment with superoxide-generating systems (unpublished results) affected *oxyR′*::*lacZ* expression. Thus, neither OxyR nor SoxRS activates the *oxyR* gene. These results and the close relation between the metabolic production of oxygen radicals and the respiratory activity of the bacterial cell (11) prompted us to evaluate the roles of the major global regulators of aerobic gene expression in *E. coli* in the control of *oxyR*. The systems examined included ArcAB, Fnr, Crp (Cap, cyclic AMP [cAMP] receptor protein) and changes in RNA polymerase σ subunits.

Anaerobic cultures of strains BGF960 (*arcB1*) and BGF970 (*arcA2*) displayed higher β-galactosidase activity than the parental strain BGF940 (Fig. 3). However, this effect may be indirect, since neither the phosphorylated or unphosphorylated form of ArcA protein bound to the *oxyR* promoter region in vitro (23). An Fnr$^-$ phenotype (strain BGF950) did not significantly alter *oxyR′*::*lacZ* expression in anaerobic cells (Fig. 3). Similar relative profiles for the various strains were observed for aerobic cultures of the strains (Fig. 3).

The presence of near-consensus half-sites (5) for Crp (TGTGA-N_6-TCAGT) in the −62 to −46 region of the *oxyR* promoter (7), together with the glucose effect reported for *katG* expression (14), prompted us to evaluate the possible effects of cAMP and Crp on *oxyR* expression. Addition of glucose (to 0.2% in the growth medium) decreased *oxyR′*::*lacZ* expression in BGF930 (*oxyR*$^+$) to ~30 U of β-galactosidase at all stages of aerobic growth. The steady-state H_2O_2 concentration was reduced in cells grown in LB medium with glucose to

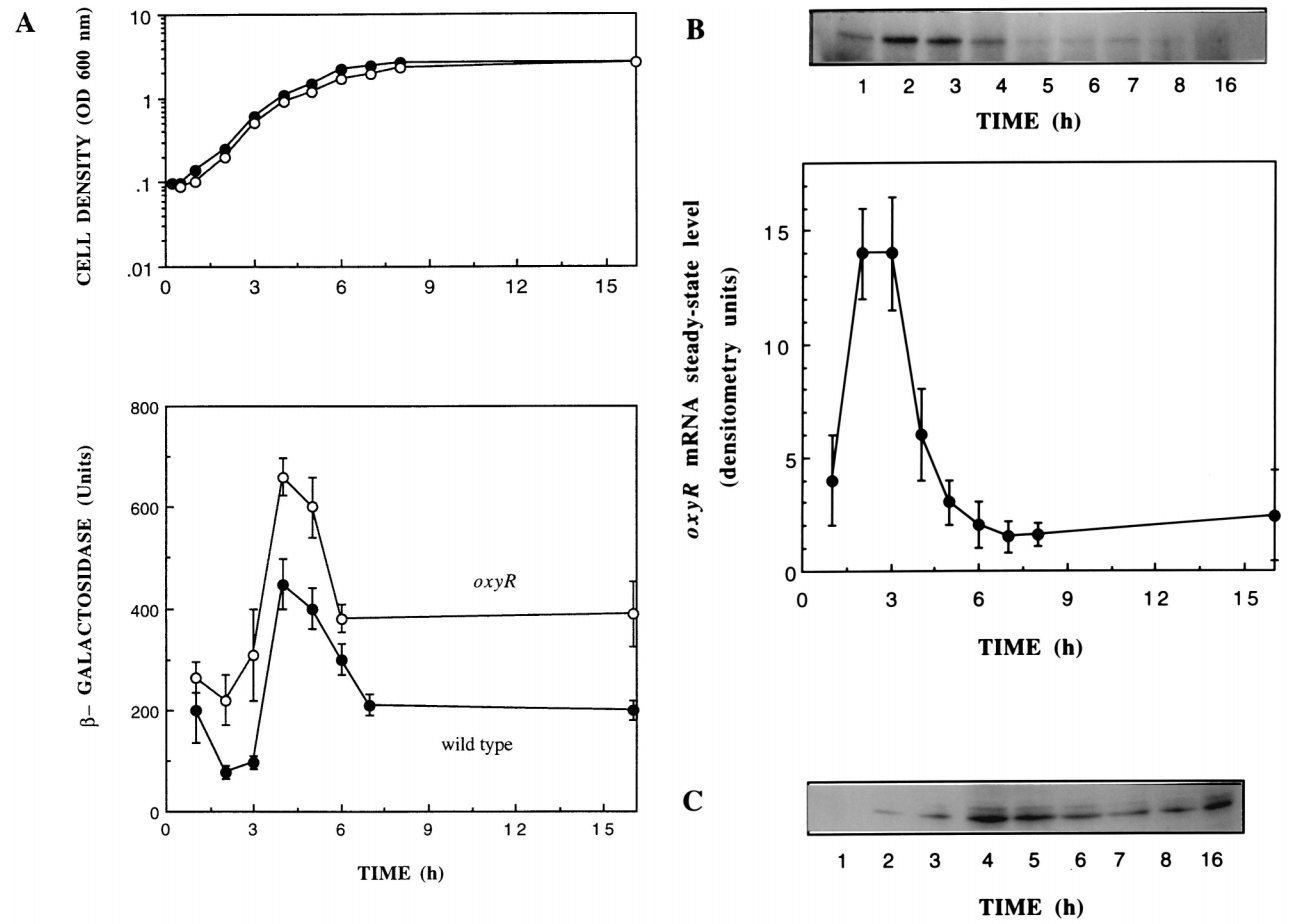

FIG. 1. Variation in *oxyR* expression during aerobic growth. (A) Expression of a single-copy *oxyR′*::*lacZ* operon fusion. Cultures of BGF930 (wild type; filled circles) and BGF932 (Δ*oxyR*; open circles) grown overnight were diluted 100-fold in fresh LB medium and incubated at 37°C with gentle shaking. At the indicated times, samples were taken to assay β-galactosidase activity (bottom). The top panel displays typical growth curves. OD 600 nm, optical density at 600 nm. (B) *oxyR* mRNA levels determined by primer extension. Strain BGF930 (*oxyR*$^+$) was grown and sampled as described above, except that the samples were processed for RNA measurements (see Materials and Methods). A representative blot (top) and densitometric quantification of three independent experiments (bottom) are shown. rRNA bands stained with ethidium bromide were used as a reference to confirm equal loading. (C) OxyR protein levels. Strain RK4936 (wild type) was grown and samples were analyzed by immunoblotting as described in Materials and Methods.

13 ± 7 nM at 2 h and 5 ± 5 nM at 6 h, which is <10% of the values found in LB medium alone (12).

The effect of glucose on *oxyR′*::*lacZ* expression was consistent with possible regulation by cAMP receptor protein. This hypothesis was tested more directly by introducing an adenylate cyclase (*cya*) mutation, which abolished expression of the single-copy *oxyR′*::*lacZ* fusion throughout the growth curve (Fig. 4A). In order to determine dependence of this effect on Crp, it was necessary to employ a multicopy plasmid containing the *oxyR′*::*lacZ* fusion (pAQ23), which was introduced into *cya* single-mutant and *cya crp* double-mutant strains. For the multicopy reporter, adenylate cyclase deficiency partially suppressed *oxyR′*::*lacZ* induction in late-exponential and stationary phases (Fig. 4B). The additional elimination of Crp almost eliminated the variation in *oxyR* expression during growth (Fig. 4B). The differences in the pattern of oscillation of *oxyR* expression observed for the multicopy plasmid in a wild-type strain (Fig. 4B) compared to that for the single-copy fusion (Fig. 1A) suggest some degree of disregulation with the multicopy reporter.

Global changes in gene expression patterns in *E. coli* can also be orchestrated by changes in RNA polymerase (26). Replacement of the σ^{70} subunit with σ^S (σ^{38}) alters RNA

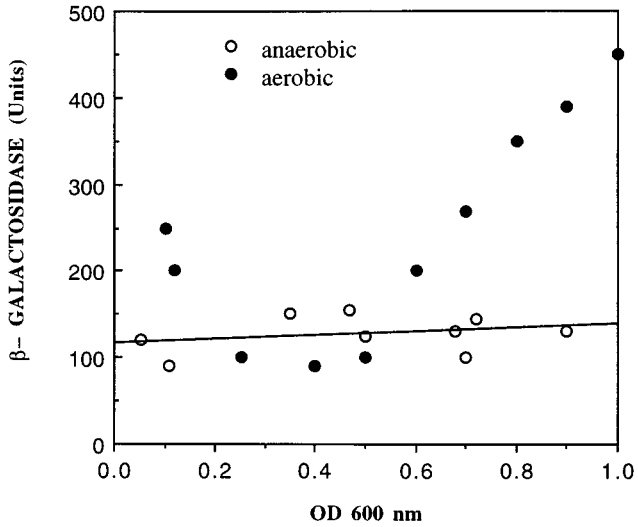

FIG. 2. Expression of a *oxyR′*::*lacZ* operon fusion in anaerobically growing *E. coli*. Cultures of strain BGF930 were grown at 37°C in 2-ml vials filled to the top with medium and fitted with butyl rubber stoppers (open circles). Data from aerobic experiments performed as described in the legend to Fig. 1 are shown for comparison. OD 600 nm, optical density at 600 nm.

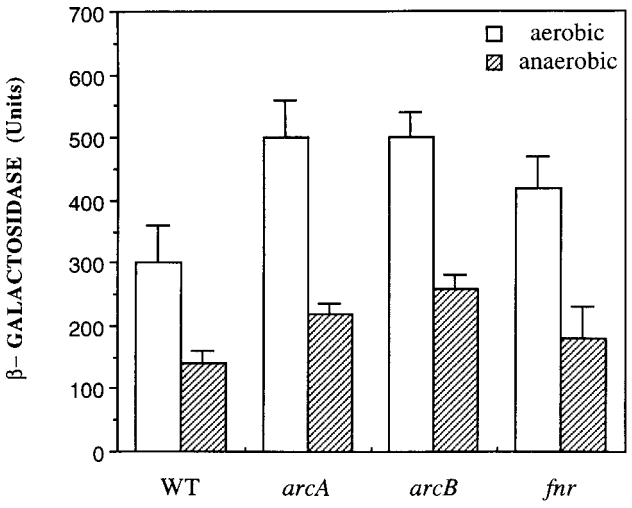

FIG. 3. Expression of a *oxyR′*::*lacZ* operon fusion in *arcAB* and *fnr E. coli*. Experimental conditions were as described in the legend to Fig. 2, with β-galactosidase activity determined at an optical density at 600 nm of 0.4 (~3 h of aerobic growth or ~16 h of anaerobic growth) for strains BGF940 (wild type [WT]), BGF950 (*fnr*), BGF960 (*arcB*), and BGF970 (*arcA*).

polymerase promoter specificity in stationary phase or upon starvation (19, 22). The *oxyR* promoter is a typical σ^{70} type (7), but the −35 consensus sequence for σ^S is not firmly established and could also be present. We therefore monitored expression of *oxyR* in an *rpoS* strain (BGF1030), a wild-type strain (BGF930), and a strain carrying a multicopy *rpoS*$^+$ (BGF930 carrying pDEB2). The expression of *oxyR* in the *rpoS* strain was the same as found for *rpoS*$^+$ cells during exponential growth but then increased steadily as the cells entered and remained in stationary phase (Fig. 5A). Thus, *oxyR* expression is maintained when there is no transition from σ^{70} to σ^S. Conversely, elevated expression of σ^S (*rpoS*) in exponential phase prevents the normal induction of *oxyR* (Fig. 5B). Elevated levels of functional σ^S in strains carrying pDEB2 are indicated by a ~10-fold-increased level of expression of the σ^S-regulated *bolA* gene during exponential growth (2).

Biological significance of the variation in OxyR levels. To address the relevance of the changes in *oxyR* expression during exponential growth, we determined the expression of two activities under *oxyR* control, catalase hydroperoxidase I (HPI), and glutathione reductase, and the susceptibility of exponentially growing bacteria to killing by H_2O_2. β-Galactosidase activity from a single-copy *katG′*::*lacZ* operon fusion and glutathione reductase enzyme activity were significantly decreased in a Δ*oxyR* strain (BGF933), as expected, and in strains with continuously reduced *oxyR* expression. Both increased *rpoS* copy number and genetic deficiency in adenylate cyclase/Crp, diminished the expression of *katG′*::*lacZ* throughout growth (approximately twofold and up to fivefold, respectively; Table 2). The sensitivity to exogenously added H_2O_2 in strains with multicopy-*rpoS*$^+$ or *cya crp* mutations was increased to the level found for a Δ*oxyR* strain (Table 2). A similar hypersensitivity to H_2O_2 (relative killing zone, 1.5 ± 0.1) was found for the Δ*cya* single mutant (strain BGF612).

DISCUSSION

The results presented here show an oscillation in *oxyR* expression during aerobic growth in *E. coli*, including a pronounced increase in OxyR during early exponential phase. This induction was eliminated by *cya cpr* mutations, which also prevented the normal elevation of catalase and glutathione reductase activities in exponential phase and increased cellular sensitivity to hydrogen peroxide. The decreased expression of *oxyR* in stationary phase could be mimicked in exponentially growing cells by increased copy number of *rpoS*, encoding stationary-phase-induced σ^S protein, which also rendered the cells more sensitive to H_2O_2. These are the first examples of variations in the expression of *oxyR* and the first indication that such changes can affect the expression of OxyR-regulated genes.

Crp protein and cAMP regulate the synthesis of many inducible enzymes in *E. coli*. Catabolite repression is mediated

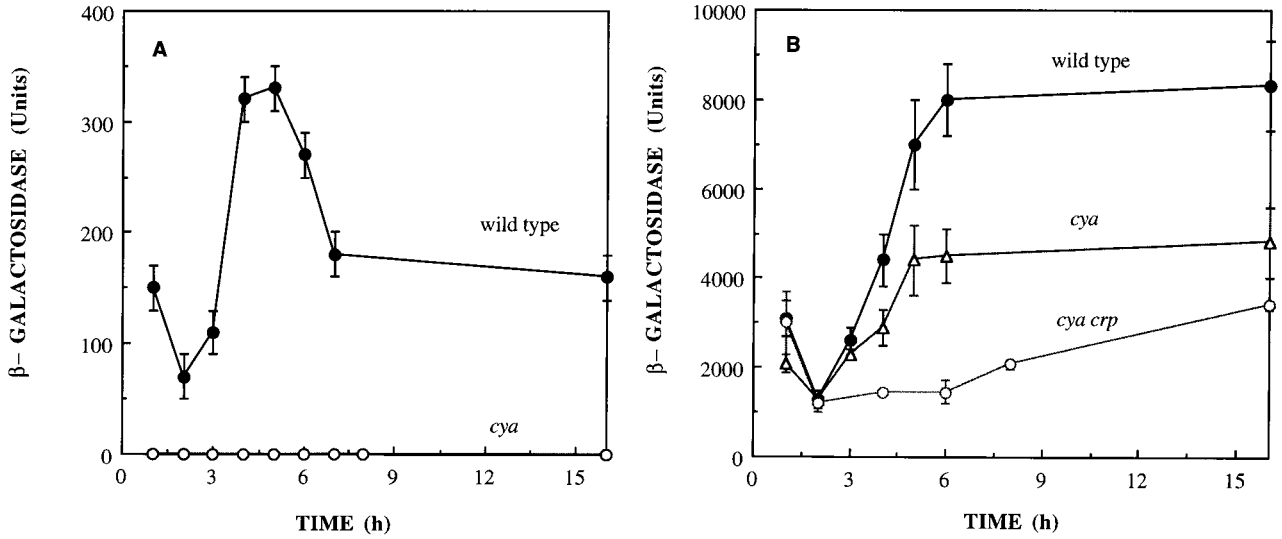

FIG. 4. (A) Expression of a single-copy *oxyR′*::*lacZ* operon fusion in an aerobically growing *E. coli cya* strain. Strains BGF930 and BGF612 were grown and sampled as described in the legend to Fig. 1. (B) Expression of a multicopy *oxyR′*::*lacZ* operon fusion in aerobically growing *E. coli cya* and *cya crp* strains. Cultures of strains CA8000 (wild type), CA8306 (*cya*), and CA8445 (*cya crp*) carrying plasmid pAQ23 (*oxyR′*::*lacZ*) grown overnight were diluted 100-fold in fresh LB broth and incubated at 37°C with shaking. Samples were taken periodically and assayed for β-galactosidase activity.

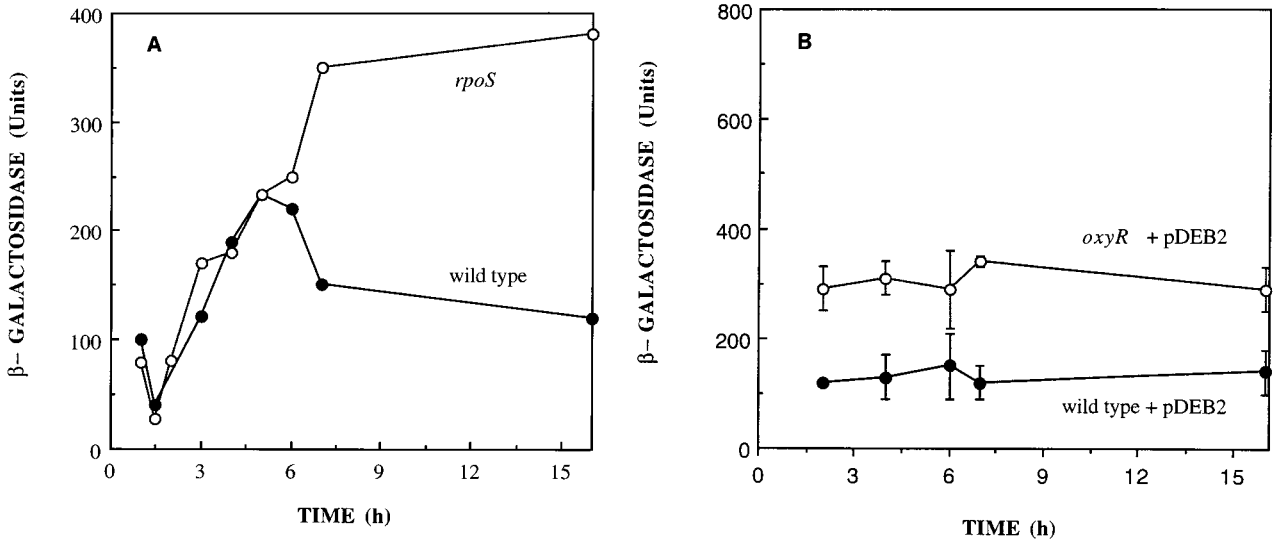

FIG. 5. Expression of a single-copy *oxyR′*::*lacZ* operon fusion in aerobically growing *E. coli* with altered *rpoS* expression. Experimental conditions were as described in the legend to Fig. 1A. (A) Strains BGF930 (*oxyR*$^+$; filled circles) and BGF1030 (*rpoS*) were grown, sampled, and assayed for β-galactosidase activity. (B) Strains BGF930 (*oxyR*$^+$) and BGF932 (*oxyR*) carrying plasmid pDEB2 (*rpoS*) were assayed for β-galactosidase expression during growth.

by a reduction in cAMP levels due to inhibition of adenylate cyclase by glucose. When the level of glucose decreases, the rise in intracellular cAMP concentration activates Crp to trigger gene expression (21). The catabolite repression reported two decades ago (14) for HPI, the product of the OxyR-dependent *katG* gene, now appears to be an indirect effect. Because the *katG* promoter has no known Crp-binding site, the Crp- or cAMP-dependent regulation of *oxyR* is the most likely mechanism to explain the glucose repression of catalase. Such indirect regulation would be expected for other OxyR-dependent genes. The loss of *oxyR* induction in a *cya crp* strain was accompanied by a 80% decrease in *katG* expression, a 55% decrease in glutathione reductase activity, and increased cellular sensitivity to H_2O_2 (Table 2), consistent with inadequate antioxidant defenses in these cells. One may speculate that catabolite repression of *oxyR* reflects the major contribution of respiration to the generation of intracellular hydrogen peroxide, the threat of which is diminished during growth in glucose.

The promoter selectivity of RNA polymerase is rapidly modulated by changes in the σ subunit (19, 22). RNA polymerase containing σ^{70} is the main form during exponential growth, while the enzyme containing σ^S increases upon some types of starvation or during the transition to stationary phase (19). The increase in the σ^S/σ^{70} ratio in stationary phase may be the key change affecting *oxyR*, since expression of *rpoS* from a multicopy plasmid also suppressed OxyR. The increased sensitivity to H_2O_2 resulting from *rpoS* overexpression is somewhat paradoxical, in that pronounced oxidant resistance is a hallmark of stationary-phase or starving *E. coli* (1, 18). It may be that the loss of the *oxyR* pathway is insufficiently compensated by the expression of *rpoS*-dependent genes under our conditions. In this regard, it should be noted that some genes (e.g., *dps*) (1) are controlled by both *oxyR* and *rpoS*.

The onset of aerobic exponential growth in *E. coli* is associated with a ~10-fold increase in the rate of H_2O_2 production from metabolic sources (11). Bacteria cope with this endogenous oxidative stress by increasing the OxyR-dependent transcription of the *katG*-encoded HPI (12). As a result of this homeostatic response, changes in the intracellular concentration of H_2O_2 are dampened to <2-fold (12). The Crp-dependent upregulation of *oxyR* reported here is evidently a key facet of the overall mechanism that acts to limit growth-dependent oxidative stress in *E. coli*. This regulation may be accounted for by the strongly increased cAMP levels reported for *E. coli* grown into stationary phase (34). Indeed, expression of *oxyR* was at least partly dependent on adenylate cyclase, although some other Crp-regulated promoters are not governed by cAMP levels (5). These include the *cst*-regulated genes which are induced in the stationary phase or under carbon starvation (5), L-asparaginase (21), and the *gyrA* gene encoding a DNA gyrase subunit (10). The elucidation of the signaling pathway that initiates induction of *oxyR* merits further study. For example, possible correlation between cAMP levels and rates of endogenous H_2O_2 generation under different growth conditions should be determined.

TABLE 2. OxyR-dependent activities and sensitivity to oxidative stress in exponential-phase cultures of strains with different levels of *oxyR* expression[a]

Strain (relevant genotype)	*oxyR′*::*lacZ*	*katG′*::*lacZ*	GSSG[d] reductase	H_2O_2 sensitivity (relative diameter of killing zone)
oxyR^{+b}	1	1	1	1
Δ*oxyR*[c]	1.5 ± 0.1	0.30 ± 0.03	0.8 ± 0.1	1.4 ± 0.1
BGF931(pDEB2) (multicopy *rpoS*)	0.27 ± 0.01	0.60 ± 0.01	0.70 ± 0.05	1.7 ± 0.1
CA800 (*cya*$^+$ *crp*$^+$)	1	1	1	1
CA8445 (Δ*cya* Δ*crp*)	0.30 ± 0.01	0.20 ± 0.01	0.45 ± 0.05	1.3 ± 0.1

[a] Cultures grown overnight were diluted 1/100 and grown aerobically for 4 h. The results (means ± standard errors) are relative to the values for the corresponding wild-type strain.
[b] *oxyR*::*lacZ* level of expression was measured in strain BGF930; *katG′*::*lacZ*, GSSG reductase, and H_2O_2 sensitivity were measured in strain BGF931.
[c] *oxyR′*::*lacZ* level of expression was measured in strain BGF932; *katG′*::*lacZ*, GSSG reductase, and H_2O_2 sensitivity were measured in strain BGF933.
[d] 6556, oxidized glutathione.

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