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Transcriptional and Posttranscriptional Regulation of Manganese Superoxide Dismutase Biosynthesis in *Escherichia coli*, Studied with Operon and Protein Fusions

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Protein and operon fusions between the manganese superoxide dismutase (MnSOD) gene, *sodA*, and genes of the lactose operon were constructed in an attempt to explore the effects of various factors on MnSOD expression and the level at which they operate. In *sodA-lacZ* protein fusions, induction of β-galactosidase perfectly mimicked MnSOD induction (i.e., β-galactosidase was not expressed in anaerobiosis and was induced by oxygen, redox-cycling compounds in aerobiosis, and iron chelators in anaerobiosis). In *tac-sodA* operon fusions, MnSOD induction was monitored only by the lactose operon inducer isopropyl-β-D-thiogalactopyranoside. Various plasmids carrying part or all of the *sodA* regulatory and structural region inhibited aerobic β-galactosidase induction in *sodA-lacZ* fusions. This included plasmids carrying only the transcription start and upstream region and also plasmids which did not contain this region and in which MnSOD was under foreign transcriptional control. The role of metal ions was also investigated. Addition of Mn(II) enhanced MnSOD activity but did not affect induction. The anaerobic expression of MnSOD from the oxygen-insensitive *tac* promoter was enhanced threefold by iron-chelating agents, implying a posttranscriptional or most likely a posttranslational modulation of enzyme activity via metal ions. To accommodate all these data, multiregulation of MnSOD is proposed.

Superoxide dismutases (SODs) are metalloenzymes of which the sole known function is to eliminate, through a dismutation reaction, the superoxide radicals produced in all organisms exposed to oxygen (13, 22).

Escherichia coli possesses two isoenzymes, an iron (3a, 45) and a manganese (27, 42)-containing SOD (FeSOD and MnSOD, respectively). Whereas FeSOD is expressed in both anaerobiosis and aerobiosis, MnSOD is present only under aerobic conditions (19). It has been shown that the aerobic induction of MnSOD occurs via superoxide radicals produced by oxidative metabolism and can be enhanced by high oxygen pressure (15) or by redox-active compounds (20, 21). Metal ions (manganese or iron) can also modify SOD activity. It has been proposed that Mn(III) and Fe(II) increase the cell content of the corresponding enzyme by speeding the conversion of the apo- to the holoenzyme (38). Furthermore, it has been reported that iron chelators induce MnSOD in both aerobic and anaerobic conditions (33, 39). These observations, made in parallel by two groups, led to different hypotheses. Moody and Hassan proposed that MnSOD is negatively regulated, at the transcriptional level, by an iron-containing repressor protein. The repressive effect depends on the ferrous state of iron in the repressor protein (33). Pugh and Fridovich suggested regulation involving autogenous repression by apo-SOD and posttranslational control based on competition between manganese and iron ions for the metal-binding site of apo-MnSOD, only the former conferring catalytic activity (39).

Global responses to different stresses, such as inhibition of DNA replication (28), treatment with alkylating agents (10), heat shock (35), and exposure to hydrogen peroxide (7), have been observed in numerous organisms and particularly in enterobacteria. Several genes whose function is to prevent, repair, or bypass the damage due to the stress are controlled by the product of a single gene, such as *recA*, *ada*, *htpR*, or *oxyR*, for the SOS, adaptive, heat shock, and oxidative responses, respectively (7, 10, 28, 35). Overlaps exist among these stress responses. Thus, some proteins induced by oxidative stress have been identified as heat shock proteins (34). Induction of MnSOD in *Escherichia coli* has been reported in *oxyR* mutants (7) or following a heat shock (37); the SOS response is evoked by elevated levels of O_2^- (3), but MnSOD induction is not part of the SOS network (16).

E. coli K-12 mutants which lack MnSOD, FeSOD, or both have been isolated (4). I describe here the construction of operon and protein fusions between MnSOD and the lactose operon genes and their use to study the effects of the different factors mentioned above on MnSOD expression. The data show a complex regulation of MnSOD which cannot be explained in its entirety by previously proposed models. The data strongly support negative transcriptional control via ferrous ions, together with posttranslational regulation of MnSOD activity depending on manganese and iron concentrations. Furthermore, to accommodate the effects in *trans* of various plasmids carrying part or all of the *sodA* structural and regulatory region, a positive transcriptional control via O_2^- and autogenous regulation are proposed. Finally, it is shown that the MnSOD gene does not belong to the *oxyR* or *htpR* regulon.

MATERIALS AND METHODS

General genetic methods (32) and standard genetic engineering procedures were as described elsewhere (30). Media and growth conditions were as described previously (43). Anaerobic conditions were obtained in a Forma Scientific anaerobic chamber (model 1024). The medium used in anaerobic experiments was first equilibrated in the chamber for 3 days. Dissolved oxygen was monitored by the oxidation of resazurin. Complete decoloration of a solution con-

TABLE 1. *E. coli* K-12 strains and plasmids used

Strain or plasmid	Relevant genotype or characteristics	Source or reference
Strains		
GC4468	F⁻ Δ*lacU169 rpsL*	R. D'Ari
QC772	GC4468 Φ(*sodA-lacZ*)*49* Cm^r Lac$^+$	4
QC774	GC4468 Φ(*sodA-lacZ*)*49* Φ(*sodB-kan*)*1*-Δ*2* Cm^r Km^r Lac$^+$	4
QC827	GC4468 Φ(*sodA-lacZ*)*71* Cm^r Lac$^+$	Fusion[a]
QC826	GC4468 Φ(*sodA-lacZ*)*76* Cm^r Lac$^+$	Fusion[a]
QC789	GC4468 (*sodB*::Mu dPR3)*1* Cm^r	4
RK4936	*oxyR*$^+$	B. Ames
TA4112	RK4936 Δ(*oxyR-btuB*)*3*	B. Ames
QC866	RK4936 Φ(*sodA-lacZ*)*49*	*sodA49* P1 transduction from QC772 into RK4936
QC878	Φ(*sodA-lacZ*)*49* Δ(*oxyR-btuB*)*3*	*sodA49* P1 transduction from QC772 into TA4412
SC122	*htpR*$^+$	8
K165	SC122 *htpR*(Am)	8
QC893	*htpR*$^+$ Φ(*sodA-lacZ*)*49*	*sodA49* P1 transduction from QC772 into SC122
QC896	*htpR*(Am) Φ(*sodA-lacZ*)*49*	*sodA49* P1 transduction from QC772 into K165
Plasmids		
pBR322	Vector, Ap^r Tc^r	41
pHC79	Vector, Ap^r Tc^r	24
pDR540	*tac* promoter source	31
pJH101	High-copy-number vector, Ap^r Tc^r Cm^r	12
pMC903	Vector, source of the *lac* fusion fragment used for detection of transcriptional initiation signals	5
pDT1-5	pHC79 carrying the *sodA*$^+$ region, Ap^r	43
pHS1-8	pBR322 carrying the *sodB*$^+$ region, Tc^r	4
pDT1-17	pHC79 carrying the *sodA* promoter region, Ap^r *sodA*	This work
pDT2-1	pDT1-5 Φ(*sodA-lacZ*)*49*	4
pDT2-6	pDT1-5 (*sodA*::Mu dPR13)*25* Ap^r Cm^r	4
pDT2-7	pDT1-5 Φ(*sodA-kan*)*49* Ap^r Km^r	This work
pDT2-8	Derived from pDT1-5; carries the *sodA* regulatory region and a truncated *sodA* structural gene cut at the *Pst*I site (bp 284)	This work
pDT1-13	Derived from pDT1-5; carries the *sodA* regulatory region and a truncated *sodA* structural gene cut at the *Nco*I site (bp 65)	This work
pDT1-10	Derived from pDT1-5; carries the COOH-terminal part of the *sodA* structural gene and the downstream region; Ap^r	This work
pDT1-11	pBR322 carrying a 1.05-kb *Ava*I fragment containing the *sodA*$^+$ region; Ap^r Tc^r *sodA*$^+$	This work
pDT1-16	pBR322 carrying an operon fusion between the *tac* promoter and the *sodA* gene; Ap^r *sodA*$^+$	This work
pDT1-21	pJH101 carrying the same fusion as pDT1-16; Ap^r *sodA*$^+$	This work
pDT1-23	Derived from pDT1-21; carries the same truncated *sodA* structural gene as pDT1-13 under *tac* promoter control; Ap^r	This work
pDT1-27	Derived from pDT1-16; carries a truncated *sodA* structural gene cut at the *Pvu*II site (bp 196) under *tac* promoter control; Ap^r	This work
pDT1-24	pDR540 carrying lactose gene under *tac* promoter control; Ap^r *lac*$^+$	This work

[a] These fusions were obtained as (*sodA-lacZ*)*49* (4).

taining 0.0002% resazurin, cysteine (500 μg/ml), and 0.5% Na_2CO_3 corresponds to a residual oxygen pressure at the threshold of detection (by measurement with an oxygen electrode) below 0.1%, as confirmed by the inability of the *Salmonella* sp. strain TA100 *uvrB* to revert on enriched minimal medium with glucose and biotin (17).

Bacteria and plasmids. The *E. coli* K-12 strains and the plasmids are listed in Table 1. See also Table 4 and Fig. 1 for plasmid representation. New plasmid constructions were as follows. pDT1-17 was obtained by *Eag*I internal deletion of pDT1-5. pDT2-7 was obtained by exchanging in pDT2-1 the *Bam*HI cassette containing the *lac* and Cm^r genes for the *Bam*HI cassette from MudPR3 containing the Km^r gene (40). pDT2-8 resulted from an internal deletion of the 7.2-kilobase (kb) *Pst*I fragment from pDT2-1, removing all the *lac* genes but retaining the Cm^r gene. pDT1-10 was obtained by a *Pvu*II internal deletion of pDT1-5. pDT1-13 was obtained by an *Nco*I-*Bam*HI deletion of pDT1-5. pDT1-11 is a subclone of the 1.05-kb *Ava*I fragment from pDT1-5 containing the *sodA*$^+$ region in the *Ava*I site of pBR322; the direction of transcription of the *sodA* gene in pDT1-11 is the same as for the *tet* gene. pDT1-16 was obtained by substitution of the *Hin*dIII-*Bam*HI fragment from pDT1-11 with the 92-base-pair (bp) *Hin*dIII-*Bam*HI fragment from pDR540 containing the *tac* promoter, followed by a *Bam*HI-*Eag*I deletion which fused the *tac* promoter to bp 27 upstream of the first ATG of the *sodA* structural gene. In a strain overexpressing the lactose repressor (*lacI*^{q} mutant) and carrying pDT1-16, MnSOD was expressed at a low level and was fully induced (10-fold) on addition of 2 mM isopropyl-β-D-thiogalactopyranoside (IPTG). pDT1-21 was constructed in the same way as pDT1-16 except that the original vector was pJH101 instead of pBR322; therefore, pDT1-21 exhibits a higher copy number than pDT1-16, as shown by its threefold-higher MnSOD expression on full IPTG induction. pDT1-23 resulted from an *Nco*I internal deletion of pDT1-21, which

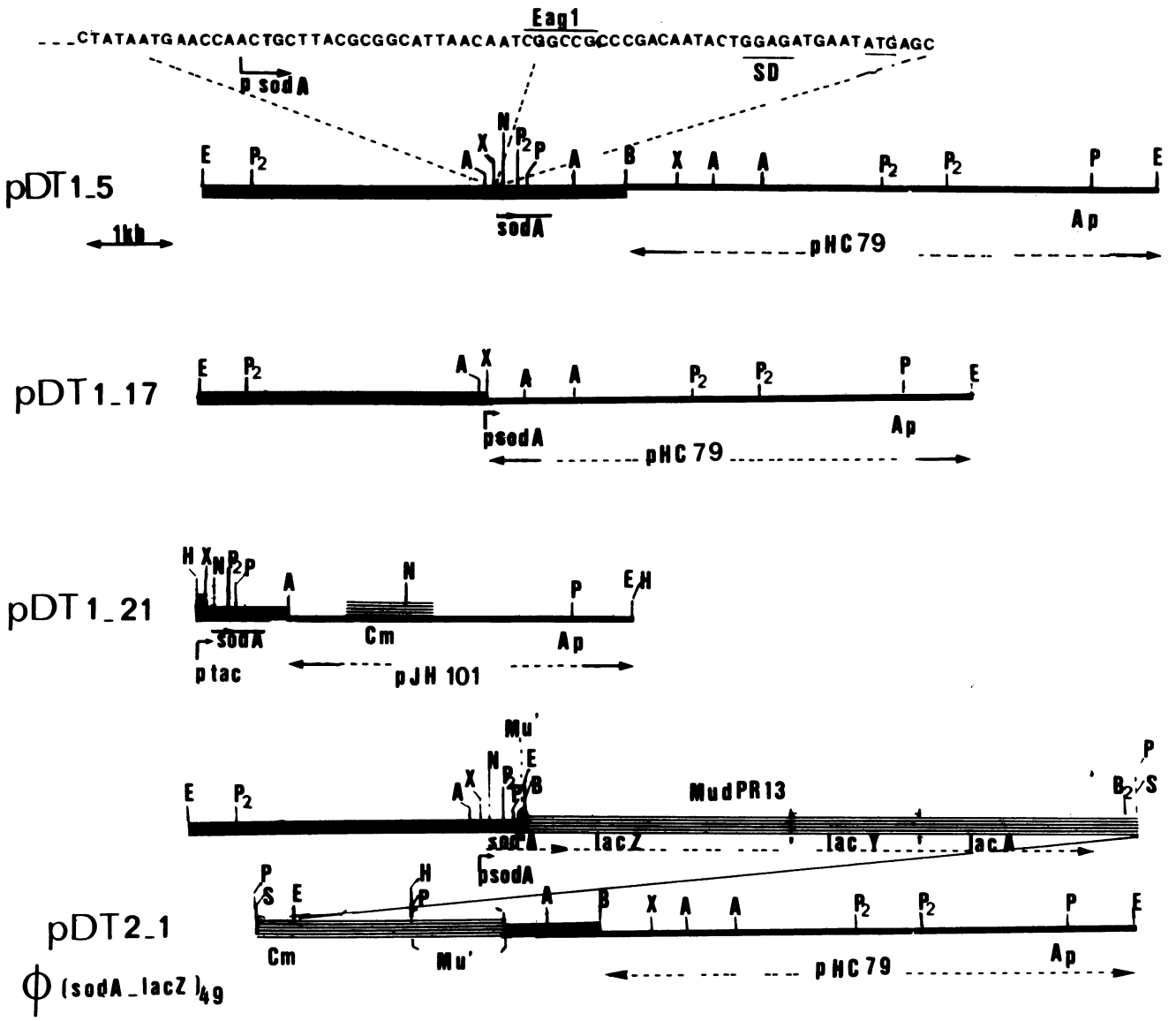


FIG. 1. Detailed restriction map of some plasmids used. Restriction endonuclease sites: E, *Eco*RI; S, *Sal*I; B, *Bam*HI; P_2, *Pvu*II; A, *Ava*I; X, *Eag*I (*Xma*I); N, *Nco*I; P, *Pst*I; B_2, *Bgl*II; H, *Hin*dIII. Heavy line, bacterial DNA; thin line, vector DNA; ruled bar, insertion.

joined the beginning of the *sodA* structural gene to the inside of the *cat* gene of pJH101. pDT1-27 was obtained by a *Pvu*II internal deletion of pDT1-16. pDT1-24 was constructed by insertion to the *Bam*HI site of pDR540 of a *Bam*HI *lac* fusion fragment from pMC903. This plasmid, in which the lactose genes are under *tac* promoter control, was used to test expression of the *tac* promoter in our various assay conditions. pDR540 was purchased from P-L Biochemicals.

Iron chelators. The iron chelators used were 1,10-phenanthroline (0.1 mM), ferrozin (0.5 mM), and 2,2-dipyridyl (0.1 mM). Depletion of intracellular iron in *E. coli* leads to siderophore induction and consequently to increased iron uptake (2). Since gallium has been reported to interfere with siderophore-mediated metal uptake (26), the addition of gallium to the medium, by competing with iron for siderophore-mediated uptake, resulted in better intracellular iron depletion by iron chelators; our control assays showed, indeed, a slight amplification of the effects of ferrous ion chelators after gallium addition and no effect of gallium by itself. Therefore, 100 μM gallium nitrate was usually added to medium for assay with iron chelators.

Crude extracts and enzyme assays. Cells were harvested and crude extracts were prepared by cycles of freezing and thawing as described previously (4), except that DNase (10 μg/ml) and $MgCl_2$ (10 mM) were added to the extraction buffer. Samples were duplicated and either used directly for enzyme measurements or loaded on small columns containing Sephadex G-25 (NAP-5; P-L Biochemicals) and eluted in 2 volumes of 50 mM phosphate buffer–0.1 mM EDTA, pH 7.8. Samples were stored at −70°C. Crude extracts from anaerobic cultures were prepared in the anaerobic chamber, except for centrifugation and freezing and thawing, which were performed in sealed tubes. Tubes containing the resazurin solution, treated in parallel with the crude extracts, were used as a control for maintenance of anaerobic conditions during extraction. Other investigators (33) have added chloramphenicol at the end of the growth period to prevent protein synthesis in the presence of air. I could not use this

procedure since the *sodA* insertion mutants are Cm^r, and puromycin, another translation inhibitor, does not completely inhibit growth in anaerobiosis, presumably due to poor penetration of the antibiotic. A similar phenomenon of apparent antibiotic resistance in anaerobiosis was also observed with kanamycin (unpublished data).

β-Galactosidase assays were done, unless otherwise stated, on whole cells grown in LB medium, as described by Miller (32). β-Lactamase assays were performed on crude extracts by the method of O'Callaghan and Morris (36) with Ceporine as the substrate (cefaloridine; Glaxo Laboratories). SOD assays were carried out as described previously (4) by the Beauchamp and Fridovich method (1). Proteins were estimated by the method of Lowry et al. (29).

RESULTS

To better understand the regulation of MnSOD, new tools were needed to discriminate between controls of MnSOD expression at the transcriptional, translational, and post-translational levels. I therefore constructed protein and operon fusions allowing us to distinguish between transcriptional and posttranscriptional events. In protein fusions, β-galactosidase is expressed from the *sodA* promoter under the transcriptional control of the *sodA* regulatory region. In such fusions, expression of β-galactosidase will not be affected by MnSOD posttranslational regulatory events. In the operon fusions, MnSOD is expressed from the *tac* promoter (9), which replaces the *sodA* promoter; the *sodA* ribosome-binding site (Shine-Dalgarno sequence) and structural gene are conserved. In such fusions, transcription of MnSOD is under *lac* repressor control and only MnSOD posttranscriptional controls will be detectable. Preliminary assays with a *tac-lacZ* fusion (pDT1-24) ensured that the *tac* promoter was insensitive to MnSOD inducers (oxygen, redox-cycling compounds, iron chelators) and normally expressed in anaerobiosis in the presence of IPTG.

Response of protein and operon fusions to MnSOD inducers. The effect of MnSOD inducers in aerobiosis and in anaerobiosis were assessed by using protein and operon fusions. Various *sodA-lacZ* fusions were constructed and introduced into the bacterial chromosome as described previously (4). Fusion sites were determined by fine restriction mapping and comparison with the published sequence (42). Three representative fusions were selected in which the hybrid gene retained about 200 bp (in *sodA76*), 380 bp (in *sodA49*), or 550 bp (in *sodA71*) of the *sodA* structural gene; none of these fusions exhibited SOD activity. No qualitative differences were observed among these fusions in the experiments reported below; therefore, data are presented only for the *sodA49* fusion. β-Galactosidase induction was generally similar in strains with or without active FeSOD (*sodA* or *sodA sodB* mutants).

Elevated oxygen concentrations induce MnSOD synthesis in *E. coli* (15); similarly, β-galactosidase synthesis was induced in a culture of the *sodA49-lacZ* strain saturated with oxygen (Fig. 2a). Increasing concentrations of paraquat, which generates O_2^- in vivo by redox cycling (20, 21), resulting in increasing differential rates of β-galactosidase synthesis (Fig. 2b). Growth was drastically inhibited at $>10^{-4}$ M paraquat.

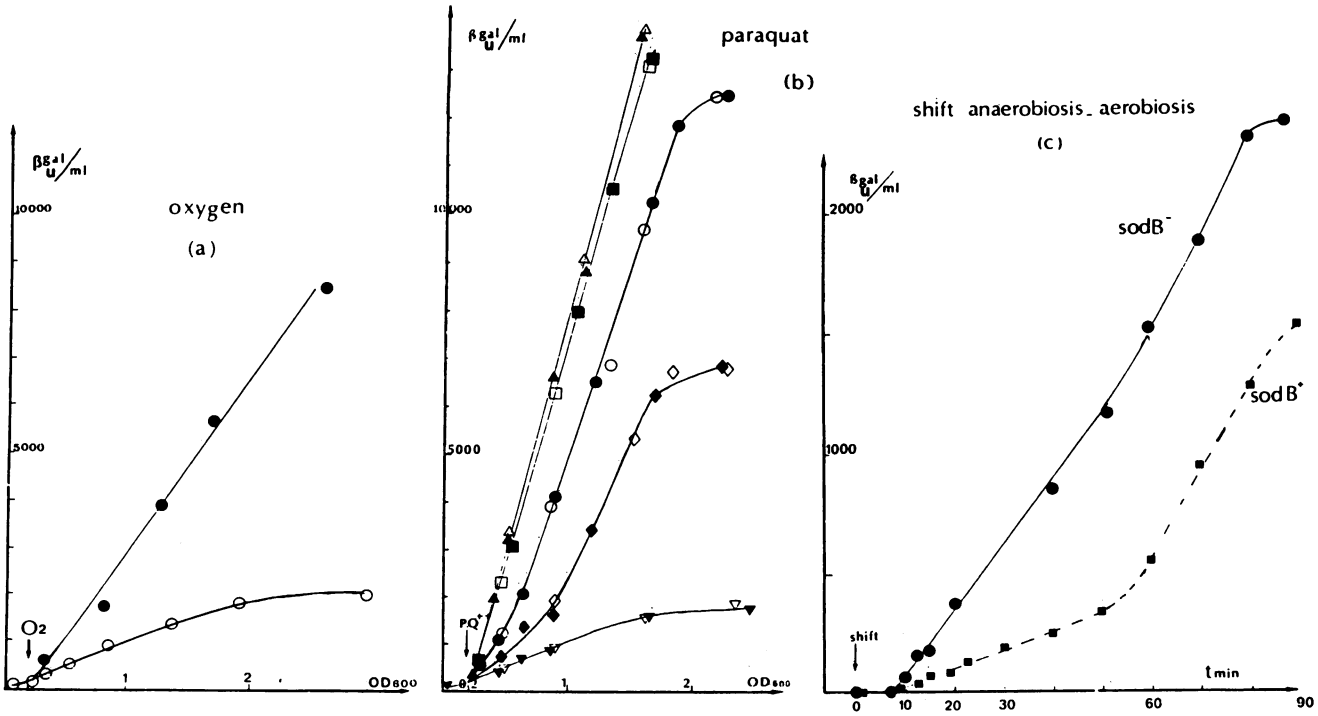


FIG. 2. Induction of β-galactosidase (βgal) in a *sodA-lacZ* fusion strain by various MnSOD inducers. (a) Effect of oxygen: oxygen (100%) was bubbled through an Erlenmeyer flask incubated at 37°C. The control was exposed to air in the rotary shaker at 200 rpm. Symbols: ●, oxygen; ○, air. (b) Effect of paraquat: paraquat (PQ^+) was added at the time indicated by the arrow. Paraquat concentrations: ▼, 0 μM; ◆, 3 μM; ●, 10 μM; ■, 30 μM; ▲, 100 μM. Open symbols represent assays with 1% glucose added to the LB medium. (c) Effect of anaerobiosis-to-aerobiosis shift. The arrows indicate the time at which 10-ml cultures in 100-ml Erlenmeyer flasks were rapidly removed from the anaerobic chamber to a rotary shaking (200 rpm) water bath in air at 37°C.

TABLE 2. Effect of various compounds on β-galactosidase induction in a *sodA-lacZ* fusion

Treatment[a]	Growth in 1 h (fold increase in OD_{600})	Differential rate[b] of β-galactosidase biosynthesis
None	5.9	870
Paraquat		
0.1 mM	5.4	8,100
0.01 mM	5.9	3,900
Plumbagin, 0.03 mM	5.0	5,700
Streptonigrin, 0.01 mM	4.2	1,550
Menadione, 0.2 mM	5.0	3,500
Methylene blue, 0.05 mM	1.7	6,300
Nitrofurantoin		
0.05 mM	2.0	700
0.005 mM	5.9	900
H_2O_2		
0.6 mM	2.1	525
0.06 mM	5.9	700
Ethanol		
2%	5.2	840
3%	4.4	820

[a] A preculture of QC772 at about 2×10^8 cells per ml was used to inoculate warm LB medium at an approximate density of 5×10^6 cells per ml. When the OD_{600} reached 0.2 (3×10^7 to 4×10^7 cells per ml), the agent to be tested was added. Samples were withdrawn at intervals for 2 h for β-galactosidase assays and OD_{600} measurements. Aeration was obtained by gyration at 200 rpm.

[b] The differential rate of β-galactosidase synthesis is expressed as the ratio of β-galactosidase increase (units per milliliter) to OD_{600} increase.

Other compounds which have been shown or are expected to produce O_2^- were tested (Table 2). As expected, plumbagin, streptonigrin, menadione, and methylene blue induced β-galactosidase, as they induced MnSOD (21). Although nitrofurantoin was reported to produce O_2^- (46), it failed to induce β-galactosidase under these conditions. This finding is in accord with previous observations by Hassan and Fridovich (21) and suggests that under these conditions nitrofurantoin does not increase O_2^- production in the cells. Streptonigrin, which strongly inhibits growth, induced β-galactosidase poorly. No significant induction was observed with H_2O_2 or ethanol. Inhibition of transcription or of translation by the addition of rifampin (50 μg/ml) or puromycin (0.5 mg/ml) suppressed β-galactosidase induction by paraquat (not shown), as it did MnSOD induction (21). Glucose has been reported to inhibit SOD biosynthesis under certain conditions, i.e., in the absence of paraquat (18). Addition of 1% glucose to the LB medium did not change β-galactosidase induction by paraquat in our experiments (Fig. 2b).

Under anaerobic growth conditions, no β-galactosidase could be detected in the *sodA-lacZ* fusion strain. β-Galactosidase was induced within 7 min after a shift of the *sodA-lacZ* mutant from anaerobiosis to aerobiosis (Fig. 2c). For about 40 min, the differential rate of synthesis was lower in the strain which had FeSOD activity than in the mutant lacking FeSOD, presumably reflecting active dismutation of the newly generated superoxide radicals by the resident FeSOD.

Addition of iron chelator to growth media has been shown to induce MnSOD in anaerobiosis (33, 39). The iron chelator 1,10-phenanthroline induced β-galactosidase equally in anaerobically grown *sodA-lacZ* and *sodA-lacZ sodB* mutants (Table 3). Similar induction, although less efficient, was obtained with two other iron chelators, ferrozin and 2,2-dipyridyl (data not shown). No further increase of induction was observed when the incubation period was prolonged, although cells were still further inducible (up to fivefold) by a shift from anaerobiosis to aerobiosis (Table 3). Whether this was the result of incomplete derepression or reflected additional regulation remains to be elucidated.

The above results show that *sodA-lacZ* fusions respond accurately to MnSOD inducers. In contrast, when the *sodA* gene was transcribed from the *tac* promoter, MnSOD induction was only under *lac* repressor control. Thus, MnSOD was induced by IPTG (data not shown), insensitive to MnSOD inducers [see QC772(pdT1-21), Table 4], and expressed in anaerobiosis [see *sodA sodB*(pdT1-16), Table 3]. This last observation supports the idea that the shut-off of MnSOD in anaerobiosis occurs primarily at the transcriptional level. These findings, together with the anaerobic induction of β-galactosidase in the *sodA-lacZ* fusion by iron chelators, are in agreement with Moody and Hassan's proposal (33) of transcriptional repression of *sodA* by an iron protein when iron is in a ferrous state.

Further support for this hypothesis was provided when ferrous ions added simultaneously with paraquat to an aerobic culture of a *sodA-lacZ* fusion strain partially inhibited induction of β-galactosidase (Fig. 3), in agreement with a previous report (33).

TABLE 3. Effect of the iron chelator 1,10-phenanthroline on MnSOD expression[a]

Strain genotype	1,10-Phenanthroline added (mM)	Anaerobiosis			Aerobiosis				
					1 h after shift from anaerobiosis			Culture in aerobiosis	
		Final OD	β-Gal[b] [U/OD (U/mg)]	SOD (U/mg)	Final OD	β-Gal [U/OD (U/mg)]	SOD (U/mg)	β-Gal [U/OD (U/mg)]	SOD (U/mg)
Φ(*sodA-lacZ*)*49*	None	1.95	52 (4.5)	12.6	2.8	5,600 (460)	15.3	5,950 (540)	14.8
	0.1	0.800	788 (108)	1.1	0.85	2,100 (285)	0.98		
sodA49 sodB(pDT1-5)[c]	None	1.7	82 (7)	1.4	2.45	4,610 (510)	115	6,300 (567)	168
	0.1	0.67	1,430 (137)	108	0.7	3,200 (420)	142		
sodA49 sodB(pDT1-16)	None	1.8	45 (3.8)	65	2.5	4,900 (440)	150	5,400 (504)	190
	0.1	0.68	1,280 (123)	175	0.75	3,200 (300)	210		

[a] Overnight cultures grown in an anaerobic chamber in LB plus 1% glucose, 100 μM $MnSO_4$, and ampicillin (when needed) (500 μg/ml) were diluted 20-fold in fresh warm medium. At OD_{600} 0.6 to 0.8, they were further diluted 10-fold in medium of the same composition containing in addition 100 μM gallium nitrate together with 100 μM 1,10-phenanthroline. Gallium nitrate was added to the control. After 3.5 h, samples were divided in two parts; one part was used for direct measurements and preparation of crude extracts, the other was transferred to aerobiosis in a 37°C water bath with vigorous gyratory shaking (200 rpm) for 1 h. Control cultures, grown in aerobiosis in identical medium, were stopped at an OD_{600} of 2.0 for measurements. Values are means of several replicate experiments, which did not differ by more than 20%. Plasmid copy number was checked by measurements of β-lactamase in each experiment.

[b] Values were from crude extracts. Values in parentheses represent measurements on whole cells, expressed in β-galactosidase (β-gal) units per OD unit as defined by Miller (32).

[c] No SOD activity was detectable in the *sodA sodB* mutant without a plasmid.

Effect of manganese and iron on MnSOD activity. Addition of manganese ions [Mn(II)] to the growth medium has been reported to increase MnSOD activity (38). In a *sodA-lacZ* fusion, induction of β-galactosidase increased with increasing paraquat concentration but remained the same on addition of Mn(II) (Fig. 3). In wild-type strains, however, induction of MnSOD activity correlated with increasing paraquat concentration only upon addition of Mn(II) (Table 4). This effect was more striking when the *sodA* gene was overexpressed from the multicopy plasmid pDT1-5. When no Mn(II) was added, no significant activity increase was observed on paraquat addition; however, induction was amplified sevenfold when 100 μM $MnSO_4$ was supplied. The same activation by Mn(II) occurred in pDT1-21, in which the *sodA* gene is transcribed from the *tac* promoter, which is insensitive to paraquat. Finally, this activation was also observed in crude extracts prepared in the presence of Mn(II) from cultures grown without added Mn(II) (data not shown). These results strongly suggest that Mn(II) activation is posttranslational and more likely reflects an intracellular deficiency of manganese ions under conditions in which there is an excess of apoenzyme, in the presence of a high level of inducer or of multiple copies of the *sodA* gene.

In anaerobiosis, the activity of SOD expressed from the *tac* promoter (in pDT1-16, Table 3) can be increased significantly (about threefold) by addition of an iron chelator. This is probably due to the ability of iron to competitively bind to the active site of MnSOD at a posttranslational level (see Discussion).

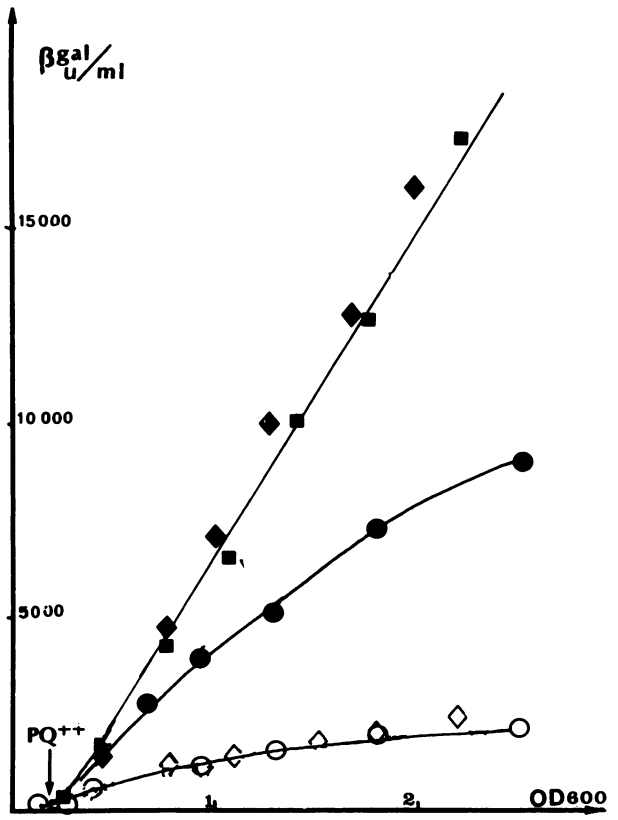


FIG. 3. Effect of metal salts on β-galactosidase (βgal) induction by paraquat in a *sodA-lacZ* fusion. Ferrous ammonium sulfate (500 μM) and manganese sulfate (500 μM) were added just before 30 μM paraquat (PQ^{++}) treatment (arrow). Symbols: ◆, no addition; ●, $FeNH_2(SO_4)_2 \cdot 6H_2O$; ■, $MnSO_4 \cdot H_2O$. Open symbols represent cultures without paraquat.

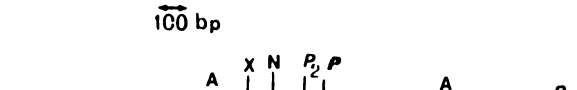
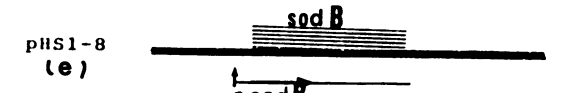
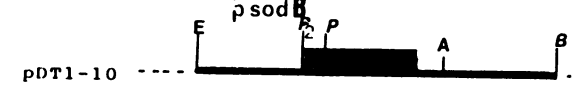
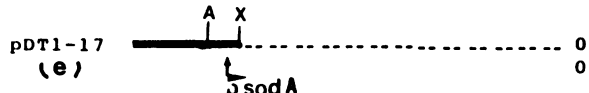
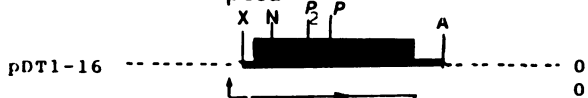
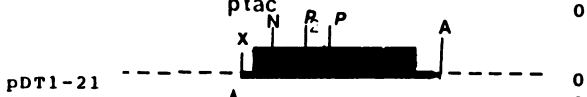
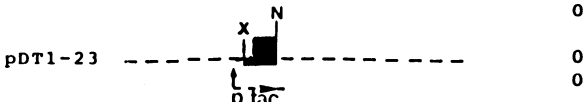
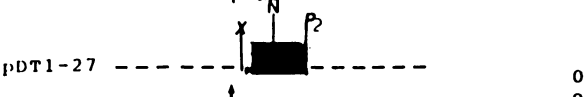
TABLE 4. Effect of Mn^{2+} on MnSOD expression and activity[a]

Strain	Paraquat concn (M)	SOD (U/mg)	
		LB	LB + 100 μM $MnSO_4$
GC4468(pBR322)	0	24	23
	3×10^{-6}	59	63
	3×10^{-5}	68	140
GC772(pDT1-5) (*sodA*)	0	87	190
	3×10^{-6}	89	340
	1×10^{-5}	91	610
	1×10^{-4}	86	1,300
QC772(pDT1-21) (*sodA*)	0	82	590
	3×10^{-6}	81	590
	1×10^{-5}	79	560
	1×10^{-4}	72	550

[a] Cultures were grown in LB medium containing ampicillin (500 μg/ml) as described in Table 2, footnote *a*. Two hours after paraquat addition, cells were chilled and used for preparation of crude extracts. Increasing the concentration of $MnSO_4$ (200 or 500 μM) gave the same results. SOD values represent total SOD activity; FeSOD activity in these experimental conditions was about 12 U/mg.

Effect in *trans* of expression of part or all of the *sodA* region on induction of β-galactosidase in a *sodA-lacZ* fusion. In a preliminary report (44), it was shown that β-galactosidase induction by paraquat in a *sodA-lacZ* fusion strain was partially inhibited when the strain harbored a multicopy plasmid (pDT1-5) carrying the *sodA* region (see Fig. 5); induction by oxygen was similarly inhibited. This effect was not the result of active dismutation of O_2^- by the excess SOD produced by the plasmid and consequent lowering of the inducer level, as shown by the following observations: (i) the same effect occurred with or without addition of Mn(II) to the growth medium, which corresponds to a 14-fold difference in SOD activity (Table 4); (ii) an identical effect was caused by plasmids carrying a truncated or disrupted *sodA* gene, devoid of SOD activity (Table 5); and (iii) plasmids like pHS1-8, carrying the *sodB* gene and expressing a high level of FeSOD activity, had no inhibitory effect on β-galactosidase induction (Table 5). Such negative effects, in *trans*, can be explained by two types of mechanisms at least: either the inhibitory effect reflects a transcriptional activator titration by the multiple copies of the regulatory region carried by the plasmid, or the inhibition is due to autogenous regulation, i.e., direct control of the protein on expression of its own structural gene (14). I therefore constructed plasmids which should allow discrimination between these two mechanisms and studied their effect on β-galactosidase induction in *sodA-lacZ* fusions (Fig. 4). The results of those studies (Table 5), discussed below, suggested that the inhibition of induction observed with pDT1-5 might actually result from regulation at both levels. The inhibition observed with plasmids containing a smaller and smaller part of the *sodA* structural gene, like pDT2-6, pDT2-8, and pDT1-13, suggested titration of a transcriptional activator. In the presence of pDT1-17, which carries only the RNA polymerase-binding site and upstream *sodA* DNA region (Fig. 1), an inhibition of induction was observed. This inhibitory effect of pDT1-17 was noticed at low levels of inducer (below 30 μM paraquat) but was no longer visible at higher concentrations of paraquat. This suggested the existence of a positive transcriptional activator that was titrated by the plasmid. However, this conclusion could not account for the inhibition at high paraquat concentrations observed with plasmids pDT1-5,

TABLE 5. Effect of expression of various plasmids on β-galactosidase induction in *sodA-lacZ* fusion strains[a]

PLASMID	PARAQUAT CONCENTRATION (mM) (b)	SOD ACTIVITY U/mg	INDUCTION RATIO (a)
	0.003	340	55 %
	0.01	610	49 %
	0.1	1300	50 %
	0.1 (c)	85	50 %
	0.003	360	100 %
	0.03	290	100 %
	0.003	15.4	100 %
	0.1	11	100 %
	0.003	15.3	58 %
	0.03	not done	62 %
	0.003	15.6	69 %
	0.01	14.3	57 %
	0.1	12.1	48.5 %
	0.003	14.8	54 %
	0.01	not done	47 %
	0.1 (d)	not done	52 %
	0.01	14.9	20 %
	0.1	not done	18 %
	0.003	15.5	60 %
	0.03	13.2	100 %
	0.03	210	53 %
	0.01	208	70 %
	0.1	196	100 %
	0.003	590	53 %
	0.01	560	56 %
	0.1	550	95 %
	0.003 (c)	81	52 %
	0.01 (c)	79	56 %
	0.003	13.9	100 %
	0.01	12.8	100 %
	0.003	14.6	100 %
	0.01	12.3	100 %

[a] Data were obtained from kinetic experiments with strain QC772, at least twice, as described in the legend to Fig. 4. $MnSO_4$ (100 μM) was added to the growth medium. The plasmid DNA fragment surrounding the *sodA* region is shown in the second column; the *sodA* structural gene is represented by a thick line and the vector by a dotted line. Underlining indicates the open reading frame corresponding to the *sodA* region, and arrows show the direction of transcription. Evidence for translation is indicated by solid underlining. Restriction sites are indicated as follows: *Nco*I, N; *Eag*I, X; *Ava*I, A; *Bam*HI, B; *Eco*RI, E; *Pvu*II, P_2; *Pst*I, P. The induction ratio is the ratio of β-galactosidase in a *sodA-lacZ* strain carrying the plasmid to the β-galactosidase in the same strain carrying a control vector, at an OD_{600} of 1. Paraquat was added between OD_{600} 0.17 and 0.20. Similar effects were observed with oxygen as an inducer (assays done with pDT1-5, pDT2-7, pDT1-21, and pHS1-8). (c) Experiment done without $MnSO_4$ addition. (d) Growth of *sodA-lacZ*(pDT1-13) was very strongly inhibited by 0.1 mM paraquat. (e) Plasmid copy number was checked as described in Materials and Methods and found to be identical to that for pDT1-5.

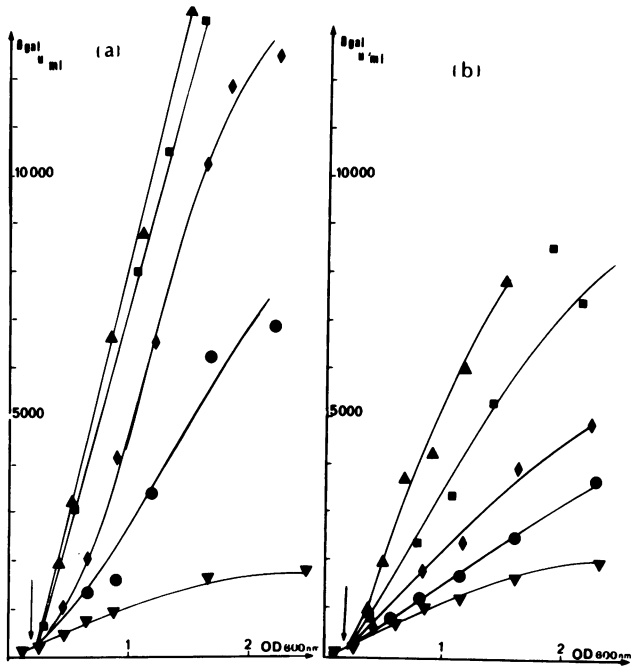


FIG. 4. Inhibition of β-galactosidase induction by paraquat in the *sodA-lacZ* strain harboring pDT1-5. Ampicillin (500 μg/ml) was added to the growth medium. The time of paraquat addition is indicated by an arrow. Paraquat concentrations: ▼, 0 μM; ●, 3 μM; ◆, 10 μM; ■, 30 μM; ▲, 100 μM. Identical induction was obtained when 100 μM $MnSO_4$ was added to the medium. Induction in (a) QC772(pHC79) and (b) QC772(pDT1-5).

pDT2-6, and others. When the symmetric experiment was performed with plasmids (pDT1-21 or pDT1-16) in which the region around the *sodA* promoter has been removed and replaced by the *tac* promoter (Fig. 1), inhibition of induction was also observed. Inhibition of induction did not depend on active enzyme and is compatible with a model of autorepression by the protein on its own biosynthesis. In favor of such a model, the increased inhibition by pDT1-21 compared with pDT1-16 correlated with the increased amount of MnSOD expressed. Also, pDT2-7, which produces a hybrid protein, had in all conditions tested (Tables 5 and 6) a stronger inhibitory effect than pDT1-5, supporting the idea of a direct interaction of the protein to control its own expression.

Plasmids carrying a truncated structural gene (pDT1-13, pDT2-6, and pDT2-8), which encode small hybrid peptides containing only the NH_2 terminus of the *sodA* gene, had the same inhibitory effect as pDT1-5, which produced the entire apoenzyme. This suggested that only the NH_2 terminus of the protein is necessary to confer regulatory ability on the peptide product encoded by the hybrid gene. The discrepancy between the effects of plasmids like pDT1-23 and pDT1-13, which carry the same *sodA* DNA fragment, might be related to the different tails of the peptide product. Indeed, those plasmids, by their construction, should encode different hybrid peptide products, as could be anticipated from the published sequence data. In construction of pDT1-13, the beginning of the *sodA* gene was fused to the middle of the *tet* gene from pBR322 (41, 42) so that 200 bp will be added to the 60 bp from *sodA* to probably form a 260-bp hybrid peptide product. In pDT1-23, the fusion to the *cat* gene carried by pJH101 (12, 42) will result in a much smaller fragment since only 18 bp could be added to the truncated *sodA* gene before a stop signal is found. This small fragment might be very unstable or even not synthesized.

TABLE 6. MnSOD induction in a *sodB* strain (QC789) carrying the pDT2-7 plasmid[a]

Strain genotype	SOD (U/mg) at paraquat concn:		
	0 M	10^{-5} M	10^{-4} M
sodB(pHC79)	17.5	106.4	176.0
sodB(pDT2-7)	18.0	22.8	27.0

[a] Growth conditions are as in Table 2, footnote *a*, except that ampicillin (500 μg/ml) and 100 μM $MnSO_4$ were added to LB medium. Two hours after paraquat addition, cells were chilled, harvested, and used for preparation of crude extracts.

Induction of MnSOD in *htpR* and *oxyR* regulatory mutants. Since MnSOD was reported to be slightly overexpressed in *oxyR* mutants (7) and by heat shock (37), I wondered whether MnSOD induction depended on heat shock or oxidative stress control, mediated by the *htpR* or *oxyR* gene. With an *sodA-lac* fusion, I measured the induction of β-galactosidase by paraquat in mutants deficient in the heat shock (*htpR*) or oxidative stress (*oxyR*) response. Induction was the same as in the corresponding parental strains, although these mutants showed high sensitivity to paraquat (Fig. 5). I conclude that induction of MnSOD is independent of those two inducible responses, as it is independent of the *recA*-dependent SOS system (16).

DISCUSSION

MnSOD was shown, several years ago, to be induced by O_2^- (15, 21). However, regulation studies always stumbled over the fact that superoxide radicals were both inducer and substrate. I therefore constructed new tools, protein fusions and operon fusions, to allow an approach in which those two functions are dissociated. In *sodA-lacZ* fusions, induction of β-galactosidase by various inducers in aerobiosis and in anaerobiosis faithfully reflected MnSOD induction. But operon fusions in which *sodA* was transcribed from the *tac* promoter were no longer inducible by superoxide radicals. This indicates that induction is a transcriptional event.

To explain the induction of MnSOD in anaerobiosis by iron chelators, Moody and Hassan (37) postulated transcriptional regulation by an iron protein which will act as a repressor when iron is in the ferrous state and will be inactive when iron is removed or oxidized. Indeed, the anaerobic induction of β-galactosidase by iron chelators in the *sodA-lacZ* fusion argues in favor of a transcriptional control via ferrous ions. Furthermore, the anaerobic presence of the multicopy plasmid pDT1-5 (*sodA*$^+$) in the *sodA-lacZ* fusion strain resulted in low expression of MnSOD and β-galactosidase (Table 3). This may indicate the titration of a transcription repressor by the multicopy plasmid, as recently reported (23). MnSOD regulation occurring via metal ions was previously emphasized (33, 39). Exchange between iron and manganese at the metal binding of MnSOD have been reported (23) in which the only manganese-charged apoenzyme is catalytically active.

In their regulation model, Pugh and Fridovich (38) proposed that addition of Mn(II) to the medium activates the conversion of MnSOD apoenzyme to holoenzyme. They also postulated a regulation of MnSOD expression based on metal (Fe and Mn) competition at the active site, depending on metal valence. The data here confirm the first proposal but do not support the general model. In anaerobiosis, active

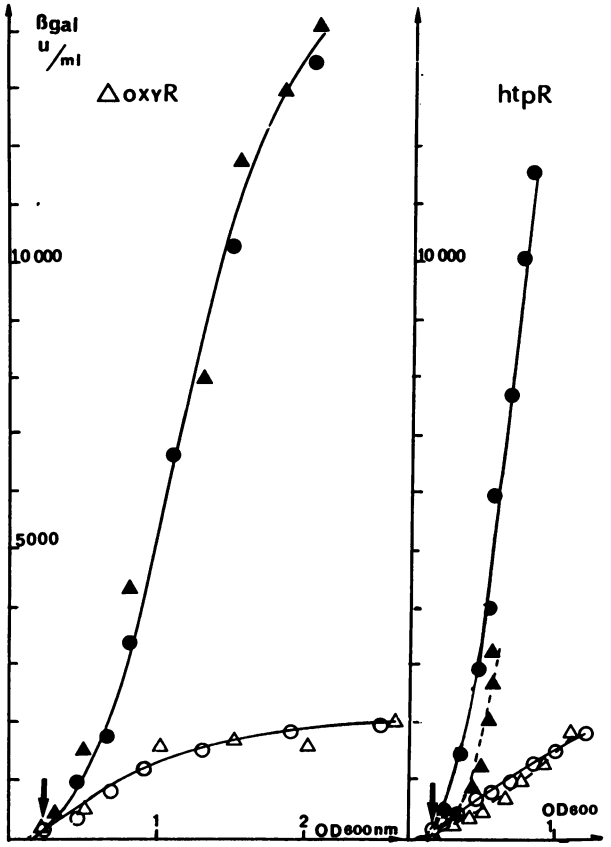


FIG. 5. Induction of β-galactosidase by paraquat in *sodA-lacZ oxyR* or *sodA-lacZ htpR* mutants. The *htpR* mutant and its parental strain were grown at 30°C. At the time indicated by an arrow, 30 μM paraquat was added. The *sodA htpR* strain grew more poorly than the corresponding *sodA* parental strain, and the growth inhibition by paraquat was stronger. Symbols: ●, parental strains (QC866 in a and QC893 in b); ▲, mutants (QC878 in a and QC896 in b). Open symbols represent paraquat-untreated cells.

MnSOD is obtained from a *tac-sodA* fusion; the further threefold enhancement by addition of iron chelators implies an inhibitory role of iron at a posttranscriptional level. This inhibitory effect presumably involves a competition between iron and manganese at the apoenzyme metal-binding site which likely depends on the intracellular concentration of manganese and iron. A similar requirement has been reported for copper addition to obtain full activity of Cu-ZnSOD in *E. coli* (34a).

Aerobic induction is partially inhibited by a plasmid which expresses MnSOD. Inhibition by plasmids carrying only the *sodA* promoter region or by plasmids which express the *sodA* gene from a foreign promoter led me to propose two levels of regulation: activation via O_2^- at the transcriptional level and autogenous regulation. These two hypotheses accommodate the data but need further studies to be supported.

Stresses often induce a defense system via a coordinate response controlled by a single gene. The data presented in this study, however, show that the control of MnSOD induction is independent of the peroxide-inducible *oxyR* regulon and of the heat shock-inducible *htpR* regulon, as it was independent of the SOS system (16). Presently, no coordinate response to the oxidative stress imposed by dioxygen or by redox-active compounds has been proved, although several findings make its existence likely. Endonuclease IV (product of the *nfo* gene) is induced by agents producing O_2^-, such as paraquat or plumbagin, or by oxygen in strains lacking SOD (6), suggesting a common regulatory pathway with MnSOD. Furthermore, cells pretreated with plumbagin or lacking SOD express a specific DNA repair system (11; S. B. Farr and D. Touati, unpublished results).

MnSOD thus appears to be multiregulated at the transcriptional and posttranscriptional levels, but I cannot say at present what the relationship is between these different controls. New tools, such as regulation mutants or in vitro studies, are a necessary next step to understanding the regulatory mechanisms for MnSOD biosynthesis.

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LITERATURE CITED

1. **Beauchamp, C., and I. Fridovich.** 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal. Biochem. **44**:276–287.
2. **Braun, V.** 1985. The unusual features of the iron transport systems of *Escherichia coli*. Trends Biochem. Sci. **10**:75–78.
3. **Brawn, M. K., and I. Fridovich.** 1985. Increased superoxide radical production evokes inducible DNA repair in *Escherichia coli*. J. Biol. Chem. **260**:922–925.

3a. **Carlioz, A., M. L. Ludwig, W. C. Stallings, J. A. Fee, H. M. Steinman, and D. Touati.** 1988. Iron superoxide dismutase: nucleotide sequence of the gene from *E. coli* K12 and correlations with crystal structures. J. Biol. Chem. **263**:1555–1562.

4. **Carlioz, A., and D. Touati.** 1986. Isolation of superoxide dismutase mutants in *E. coli*: is superoxide dismutase necessary for aerobic life? EMBO J. **5**:623–630.
5. **Casadaban, M. J., J. Chou, and S. N. Cohen.** 1980. In vitro gene fusions that join an enzymatically active β-galactosidase segment to amino-terminal fragments of exogenous proteins: *Escherichia coli* plasmid vectors for the detection and cloning of transcriptional initiation signals. J. Bacteriol. **143**:971–980.
6. **Chan, E., and B. Weiss.** 1987. Endonuclease IV of *E. coli* is induced by paraquat. Proc. Natl. Acad. Sci. USA **84**:3189–3193.
7. **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.
8. **Cooper, S., and T. Ruettinger.** 1975. A temperature sensitive nonsense mutation affecting the synthesis of a major protein of *Escherichia coli* K12. Mol. Gen. Genet. **139**:167–176.
9. **De Boer, H. A., L. J. Comstock, and M. Vasser.** 1983. The *tac* promoter: a functional hybrid derived from the *trp* and *lac* promoters. Proc. Natl. Acad. Sci. USA **80**:21–25.
10. **Demple, B., Y. Daikh, J. Greenberg, and A. Johnson.** 1986. Alkylation and oxidative damages to DNA: constitutive and inducible repair systems, p. 205–217. *In* D. M. Shankel, P. E. Hartman, T. Kada, and A. Hollaender (ed.), Antimutagenesis and anticarcinogenesis mechanisms. Plenum Publishing Corp., New York.
11. **Farr, S. B., D. O. Natvig, and T. Kogoma.** 1985. Toxicity and mutagenicity of plumbagin and the induction of a possible new DNA repair pathway in *Escherichia coli*. J. Bacteriol. **164**:1309–1316.
12. **Ferrari, F. A., A. N. Guyen, D. Lang, and J. A. Hoch.** 1983. Construction and properties of an integrable plasmid for *Bacillus subtilis*. J. Bacteriol. **154**:1513–1515.
13. **Fridovich, I.** 1986. Superoxide dismutases. Adv. Enzymol. **58**: 68–97.
14. **Goldberger, R. F.** 1974. Autogenous regulation of gene expression. Science **183**:810–816.
15. **Gregory, E. M., and I. Fridovich.** 1973. Induction of superoxide

dismutase by molecular oxygen. J. Bacteriol. **114**:543–548.
16. **Hancock, L. C., and H. M. Hassan.** 1985. Regulation of the manganese containing superoxide dismutase is independent of the inducible DNA repair system in *E. coli*. J. Biol. Chem. **260**:12954–12956.
17. **Hartman, Z., P. E. Hartman, W. M. Barnes, and E. Tuley.** 1984. Spontaneous mutation frequencies in *Salmonella*: enhancement of G/C to A/T transitions and derepression of deletion and frameshift mutation frequencies afforded by anoxic incubation. Environ. Mutagen. **6**:633–650.
18. **Hassan, H. M., and I. Fridovich.** 1977. Regulation of superoxide dismutase synthesis in *Escherichia coli*: glucose effect. J. Bacteriol. **132**:505–510.
19. **Hassan, H. M., and I. Fridovich.** 1977. Enzymatic defense against the toxicity of oxygen and of streptonigrin in *Escherichia coli*. J. Bacteriol. **129**:1574–1583.
20. **Hassan, H. M., and I. Fridovich.** 1977. Regulation of the synthesis of superoxide dismutase in *E. coli* induction by methyl viologen. J. Biol. Chem. **252**:7667–7672.
21. **Hassan, H. M., and I. Fridovich.** 1979. Intracellular production of superoxide radical and of hydrogen peroxide by redox active compounds. Arch. Biochem. Biophys. **136**:385–395.
22. **Hassan, H. M., and I. Fridovich.** 1980. Superoxide dismutases: detoxication of a free radical, p. 311–322. *In* W. B. Jacoby (ed.), Enzymatic basis of detoxication, vol. 1. Academic Press, New York.
23. **Hassan, H. M., and C. Moody.** 1987. Regulation of manganese-containing superoxide dismutase in *E. coli*. Anaerobic induction by nitrate. J. Biol. Chem. **262**:17173–17177.
24. **Hohn, B., and J. Collins.** 1980. A small cosmid for efficient cloning of large DNA fragments. Gene **11**:291–298.
25. **Horinouchi, S., and B. Weisblum.** 1982. Nucleotide sequence and functional map of pC194, a plasmid that specifies inducible chloramphenicol resistance. J. Bacteriol. **150**:815–825.
26. **Hubbard, J. A. M., K. B. Lewandowska, M. N. Hugles, and R. K. Poole.** 1986. Effects of iron limitation of *Escherichia coli* on growth, the respiratory chains and gallium uptake. Arch. Microbiol. **146**:80–86.
27. **Keele, B. B., Jr., J. M. McCord, and I. Fridovich.** 1970. Superoxide dismutase from *E. coli* B: a new manganese containing enzyme. J. Biol. Chem. **245**:6176–6181.
28. **Little, J. W., and D. W. Mount.** 1982. The SOS regulatory system of *Escherichia coli*. Cell **29**:11–22.
29. **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.
30. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
31. **McKenney, K., H. Shimatak, D. Court, U. Schmeissner, C. Brady, and M. Rosenberg.** 1981. A system to study promoter and terminator signals recognized by *Escherichia coli* RNA polymerase, p. 383–415. *In* J. G. Chirikjian and T. S. Papas (ed.), Gene amplification and analysis, vol. 2: analysis of nucleic acids by enzymatic methods. Elsevier/North-Holland Publishing Co., Amsterdam.
32. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
33. **Moody, C. S., and H. M. Hassan.** 1984. Anaerobic biosynthesis of the manganese containing superoxide dismutase in *E. coli*. J. Biol. Chem. **259**:12821–12825.
34. **Morgan, R. W., M. F. Christman, F. S. Jacobson, G. S. Storz, and B. N. Ames.** 1986. Hydrogen peroxide inducible proteins in *Salmonella typhimurium* overlap with heat shock and other stress proteins. Proc. Natl. Acad. Sci. USA **83**:8059–8063.
34a. **Natvig, D. O., K. Imlay, D. Touati, and R. Hallewell.** 1987. Human copper-zinc superoxide dismutase complements superoxide dismutase deficient *E. coli* mutants. J. Biol. Chem. **262**:14697–14701.
35. **Neidhardt, F. C., R. A. VanBogelen, and V. Vaughn.** 1984. The genetics and regulation of heat shock proteins. Annu. Rev. Genet. **18**:295–329.
36. **O'Callaghan, C. H., and A. Morris.** 1972. Novel method for detection of β-lactamase by using a chromogenic cephalosporine substrate. Antimicrob. Agents Chemother. **1**:283–288.
37. **Privalle, C. T., and I. Fridovich.** 1987. Induction of superoxide dismutase in *E. coli* by heat shock. Proc. Natl. Acad. Sci. USA **84**:2723–2726.
38. **Pugh, S. Y. R., J. L. DiGuiseppi, and I. Fridovich.** 1984. Induction of superoxide dismutase in *Escherichia coli* by manganese and iron. J. Bacteriol. **160**:137–142.
39. **Pugh, S. Y. R., and I. Fridovich.** 1985. Induction of superoxide dismutase in *Escherichia coli* B by metal chelators. J. Bacteriol. **162**:196–202.
40. **Ratet, P., and F. Richaud.** 1986. Construction of a new transposable element whose insertion is able to produce gene fusion with noemycin phosphotransferase coding region of Tn903. Gene **42**:185–192.
41. **Sutcliffe, J. G.** 1979. Complete nucleotide sequence of the *Escherichia coli* plasmid pBR322. Cold Spring Harbor Symp. Quant. Biol. **43**:77–90.
42. **Takeda, Y., and H. Avila.** 1986. Structure and gene expression of the *E. coli* Mn superoxide dismutase gene. Nucleic Acids Res. **11**:4577–4589.
43. **Touati, D.** 1983. Cloning and mapping of the manganese superoxide dismutase gene (*sodA*) of *Escherichia coli* K-12. J. Bacteriol. **155**:1078–1085.
44. **Touati, D., and A. Carlioz.** 1986. Superoxide dismutase mutants of *E. coli*, p. 287–292. *In* G. Rotilio (ed.), Superoxide and superoxide dismutase in chemistry, biology and medicine. Elsevier/North-Holland Publishing Co., Amsterdam.
45. **Yost, F. J., Jr., and I. Fridovich.** 1973. An iron containing superoxide dismutase from *E. coli*. J. Biol. Chem. **248**:4905–4908.
46. **Youngman, R., W. F. Oswald, and E. F. Elstner.** 1982. Crypto-OH radical production by nitrofurantoin. Biochem. Pharmacol. **31**:3723–3729.