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Regulation of the *sufABCDSE* Operon by Fur

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A promoter that is inducible by paraquat and menadione, the superoxide generators, independently of *soxRS* has been found in front of the *sufABCDSE* operon in *Escherichia coli*. Based on the observation that SufA is a holomog of IscA that functions in the assembly of iron sulfur cluster and the *sufA* promoter (*sufA*p) contains a putative Fur-binding consensus, we investigated whether this gene is regulated by Fur, a ferric uptake regulator. When examined in several *sufA*p-*lacZ* chromosomal fusion strains, *sufA*p was induced by EDTA, an iron chelator and a well-known Fur-inducer. The basal level of *sufA* expression increased dramatically in *fur* mutant, suggesting repression of *sufA*p by Fur. The derepression in *fur* mutant and EDTA-induction of *sufA* expression required nucleotides up to -61, where a putative Fur box is located. Purified Fur protein bound to the DNA fragment containing the putative Fur box between -35 and -10 promoter elements. The regulation by Fur and menadione induction of *sufA*p acted independently. The *rpoS* mutation increased *sufA* induction by menadione, suggesting that the stationary sigma factor RpoS acts negatively on *sufA* induction.

Key words: Fur, superoxide, iron, SoxRS, oxidant induction, RpoS

In *Escherichia coli*, more than 40 proteins are induced by the superoxide-generating agents, such as paraquat and menadione (Greenberg and Demple, 1989; Walkup and Kogoma, 1989). Recent analyses using microarray demonstrated induction of more than 100 genes in response to paraquat (Pomposiello *et al.*, 2001). Only a part of them have been characterized at the gene or protein level, most of which belong to *soxRS*-regulon. Evidences for the presence of other regulatory system than *soxRS* against redox cycling agents have accumulated. For example, quite a few uncharacterized protein spots are induced or repressed by superoxide generating agents independently of *soxRS* locus in 2-D gel analysis (Greenberg and Demple, 1989; Greenberg *et al.*, 1990; 1991). The *hmp* gene encoding flavohemoglobin is induced by paraquat and nitric oxide, independently of SoxRS (Poole *et al.*, 1996; Membrillo-Hernàndez *et al.*, 1997). Similarily, the *mutM* gene encoding 8-hydroxyguanine endonuclease is positively regulated by superoxide generating agents independently of SoxRS (Lee *et al.*, 1998). Although some regulators such as Fur (ferric uptake regulation) and Fnr (fumarate nitrate reductase) have been suggested as the responsible sensor proteins, the identity of regulators and their mechanism of action await further elucidation.

Previously we found that a promoter, HC96, is induced by paraquat and menadione independently of SoxRS (Lee and Roe, 1997). HC96 promoter was located immediately upstream of six consecutive open reading frames which were named recently as *sufABCDSE* genes. These genes are cotranscribed from one start site, suggesting they constitute an operon. The *suf* genes are present in a number of species including bacteria, Archaea, plants and parasites, especially *sufB*, *C* and *D* are well conserved in those species. SufA is an IscA homolog, which is known to be involved in [Fe-S] cluster formation and repair together with IscSU (a NifSU-homolog) (Rangachari *et al.*, 2002). SufA is also predicted to belong to the *hesB/yadR/yfhF* family of proteins. It has been shown that *E. coli* IscA improves the efficiency of the maturation of overexpressed ferredoxins within *E. coli* cells (Takahashi and Nakamura, 1999). Moreover, in *Saccharomyces cerevisae*, two strong IscA homologs and mitochondrial proteins Isa1p and Isa2p were shown to be important for normal mitochondrial and cytoplasmic iron metabolism and to play a crucial role in the maturation of iron-sulfur proteins (Jensen and Culotta, 2000; Kaut *et al.*, 2000; Pelzer *et al.*, 2000). Recently, it has been reported that SufA from *Erwinia chrisanthemi* is located in the cytosol and serves not only as a [2Fe-2S] cluster donor to ferredoxin but also as a [4Fe-4S] donor for biotin synthase, BioB, *in vitro* (Ollagnier-De Choudens *et al.*, 2003). SufBCD are homologs of ABC transporter components, similar to MDR (multidrug resistance) proteins for drug efflux. It has been demonstrated that they all exist in the cytosol

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and SufC interacts with both SufB and SufD to function as an unorthodox cytoplasmic ABC ATPase as a complex in *E. chrisanthemi* (Nachin *et al.*, 2003). But their role in [Fe-S] cluster assembly is still dubious and one possible role would be to provide energy to the Suf machinery for [Fe-S] cluster assembly. SufS is a homolog of NifS, which has been demonstrated to be a desulfurase (Flint, 1996; Mihara *et al.*, 1997; Fujii *et al.*, 2000) and may provide sulfur to the [Fe-S] cluster.

Recently, the *suf* genes were found to be regulated by OxyR in response to H_2O_2 (Zheng *et al.*, 2001), and in oxidative stress condition *suf* mutant is more sensitive to paraquat with more iron accumulated, compared with the wild type cell (Nachin *et al.*, 2001). And also the activity of [Fe-S] proteins was decreased by treatment of oxidants in *sufC* mutant, suggesting a critical role of the Suf machinery under oxidative stress conditions (Nachin *et al.*, 2003). The Fur-dependent expression of *sufD-lacZ* and *sufS-lacZ* fusion genes has been demonstrated in *E. coli*, suggesting that the *suf* operon is under negative regulation of Fur (Patzer and Hantke, 1999). In *E. chrysanthemi*, a plant pathogen, expression of *sufB-uidA* fusion gene is under the control of Fur (Nachin *et al.*, 2001). Even though the presence of a putative Fur-binding site is predicted within the promoter region, no direct experimental observation with the *sufA* promoter has been presented so far. Here, we report on the regulation of *sufA* transcription by Fur *in vivo* and *in vitro*. We also report on the separate action of Fur regulation from oxidant-induction of the *sufA* gene.

Materials and Methods

Strains, phages, and plasmids

Bacterial strains, phages and plasmids used in this study are listed in Table 1. *E. coli* strain DH5α was used as a host to construct the recombinant DNAs. The promoter probing vector pRS415, which contains promoterless *lacZ* gene and the ampicillin resistance marker, was used to construct *lacZ* reporter fusion and to deliver *sufA*p-*lacZ* fusion genes into the chromosome (Simons *et al.*, 1987).

Growth media and culture conditions

LB medium (1% tryptone, 1% NaCl, and 0.5% yeast extracts) was used for routine bacterial culture. Antibiotics were used at the following concentrations; ampicillin (50 μg/ml), kanamycin (25 μg/ml), tetracycline (15 μg/ml), and chloramphenicol (20 μg/ml). For the treatment of the redox-cycling agents or EDTA, cells were grown in LB up to OD_{600}=0.2 and treated with the agents at various concentrations for 1 h.

Table 1. Bacterial strains and plasmids used in this study

	Description	Source or Reference
Strains		
DH5α	*supE44 lacU169 (80 lacZ M15) hsdR17 recA1 endA1 gyrA96 thi-relA1*	Sambrook *et al.* (1989)
GC4468	*(argF-lac)169 rpsL sup*(Am)	Touati (1983)
BW829	GC4468, *Δsox-8*::*cat*[a], Chl[r]	Tsaneva and Weiss (1990)
KT1100	MC4100, *rpoS*::Tn*10*, Tet[r]	lab. collection
NC442	GC4468, *fur*::Tn*5kan*, Kan[r]	Lee *et al.* (1998)
SPD337	-337 ~ +90 *suf* promoter-*lacZ* fusion	This work
SPD256	-256 ~ +90 *suf* promoter-*lacZ* fusion	This work
SPD184	-184 ~ +90 *suf* promoter-*lacZ* fusion	This work
SPD112	-112 ~ +90 *suf* promoter-*lacZ* fusion	This work
SPD61	-61 ~ +90 *suf* promoter-*lacZ* fusion	This work
Plasmid		
pRS415	*lacZYA* operon fusion vector, Amp[r]	Simons *et al.* (1987)
pET3a	protein overexpression vector, Amp[r]	Novagen
pET3a-*fur*	*fur* in pET3a	This work
Phages		
λRZ5	Φ(*bla'-'lacZ*)*lacY*[+]	Simons *et al.* (1987)
$P1_{vir}$	virulent derivative of P1 phage	Silhavy *et al.* (1984)

[a]*sox-8*::*cat* is a deletion mutation of *soxR* and *soxS*.

Recombinant DNA techniques

Restriction enzymes, Klenow fragment of *E. coli* DNA polymerase I, calf intestine alkaline phosphatase, T4 polynucleotide kinase, Taq DNA polymerase, and T4 DNA ligase were purchased from POSCOCHEM, Stratagene, Takara, or Promega Corporation. All the chemicals used in this study were of reagent or molecular biology grade. Standard recombinant DNA techniques were employed as described by Sambrook *et al.*, (1989) or according to recommendations by the manufacturer.

Construction of lacZ fusion strains

In order to construct strains containing single copy *sufAp-lacZ* fusions in the chromosome, we followed the procedure developed by Simons *et al.* (1987). Various lengths of *sufA* upstream region were ligated to the promoterless *lacZ* gene in pRS415, and then transferred onto λRZ5 by homologous recombination *in vivo*. The recombinant phages lysogenized *E. coli* GC4468 via recombination through the *att* site in the chromosome. Single copy lysogens were further selected on the basis of pale blue color of the ampicillin-resistant colonies on the X-Gal plate, and further confirmed for the lowest basal level of β-galactosidase activity.

Construction of mutant strains

$P1_{vir}$, a transducing phage, was used to create mutant strains of various genetic backgrounds according to the standard procedures (Miller, 1972; Silhavy *et al.*, 1984). $P1_{vir}$ lysates containing various mutant alleles, such as *Δsox-8*::*cat* (from BW829), *rpoS*::Tn*10* (from KT1100), and *fur*::Tn*5kan* (from NC442), were prepared from infecting original mutant strains (Table 1) by plate lysate method. The lysates containing the phages (about 10^9 pfu) were transduced into appropriate host strains. The transductants were selected by the antibiotic resistance marker associated with the mutation (chloramphenicol for *Δsox-8*::*cat*, tetracycline for *rpoS*::Tn*10*, and kanamycin for *fur*::Tn*5kan*).

β-galactosidase assay

β-galactosidase activity was assayed in whole cells using ONPG (o-nitrophenyl-β-D-galactopyranoside) as a substrate after permeabilization of cells with SDS-chloroform (Miller, 1972). Cells were grown in LB up to OD_{600}=0.2 and either untreated or treated with various concentrations of menadione or EDTA for 1 h at 37°C.

Purification of Fur

Fur protein was purified as described by Althaus *et al.* (1999). The entire coding region of the *fur* gene was amplified by PCR, cloned into the overexpression vector, pET3a (pET3a-*fur*), and introduced into *E. coli* strain BL21 (DE3). Transformants were grown in LB broth containing ampicillin (50 μg/ml) and chloramphenicol (34 μg/ml) at 37°C to OD_{600}=0.5 and further incubated with 1 mM IPTG at 18°C for 14 h. Cells were harvested, resuspended in 50 mM Tris-Cl (pH 8.0), and disrupted by French Press. Following centrifugation, the supernatant was precipitated with 80% ammonium sulfate and the pellet was dissolved in 50 mM Tris-Cl (pH 8.0) and applied onto the Zn-IDA column (1.6 cm×20 cm) equilibrated with 50 mM Tris-Cl (pH 8.0) containing 500 mM $ZnCl_2$. The column-bound proteins were washed with 50 mM Tris-Cl (pH 8.0) and were eluted using linear gradient of histidine from 0 to 50 mM. Fur was eluted at 10-25 mM histidine. The fractions containing purified Fur were visualized on 15% SDS-PAGE, pooled, and dialyzed in the storage buffer [50 mM Tris-Cl (pH 8.0), 50% glycerol, 200 mM NaCl). The concentration of purified Fur was determined by the Bradford method with BSA as a standard.

Gel mobility shift assay

151 bp DNA fragment from -61 to +90 nucleotides relative to the transcription start site (+1) of the *sufA* gene was amplified by PCR, and labeled with [γ-^{32}P] ATP by T4 polynucleotide kinase. The labeled fragment was digested with *Cla*I to produce two fragments, which were incubated with increasing amounts of purified Fur at 30°C for 15 min in the binding buffer. The complexes were resolved on 5% polyacrylamide gel by electrophoresing through TAE buffer [20 mM Tris-HCl (pH 8.0), 3 mM sodium acetate (pH 7.9), 1 mM EDTA] at 180 V for 4-5 h. The binding buffer (20 μl) contained 10 mM Tris-Cl (pH 7.5), 1 mM $MgCl_2$, 40 mM KCl, 0.1 mM $MnSO_4$, 100 ng/ml poly(dI-dC), and 5% glycerol. The gel was dried and visualized by autoradiography.

Results

Regulation of sufA promoter by Fur

The *sufA* promoter *(sufA*p) contains a well-conserved Fur-binding motif between -35 and -10 promoter elements (Fig. 1). To investigate the regulation of the *suf* operon and experimentally localize the responsible regulatory elements, we constructed various *sufA*p-*lacZ* fusion strains containing different lengths of *sufA* upstream region up to -337, -256, -184, -112, and -61 nucleotides from the transcription start site (Fig. 1A). When these cells were treated with EDTA, an iron-chelator, *sufA-lacZ* expression was induced more than 6-fold (Fig. 2A). Consistently, $FeCl_3$ repressed *sufA* expression by 70% (data not shown). The shortest promoter fragment (-61) was successfully induced, implying that EDTA-induction requires a region between -61 and +90.

To verify whether Fur is responsible for EDTA-induction, we introduced a *fur*-mutation (*fur*::Tn*5kan*) into the *sufA-lacZ* fusion strain. As demonstrated in Fig. 2B, the basal expression level of *sufA* in the *fur* mutants was

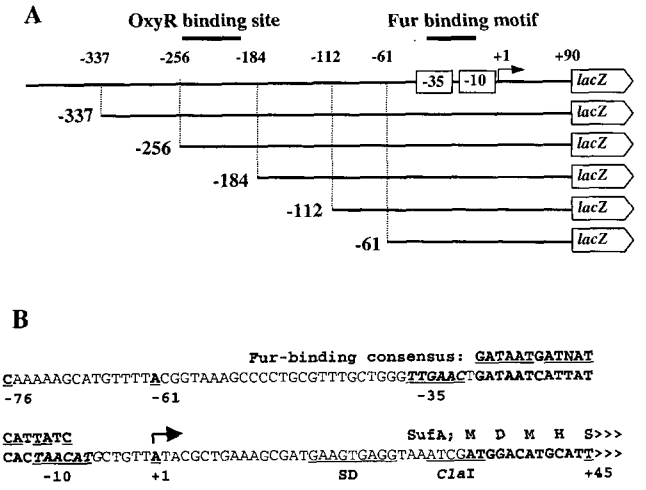

Fig. 1. The structure of various *sufA-lacZ* fusions and *sufA* promoter region. Various *sufA-lacZ* fusion constructs used in this study are demonstrated (A). The OxyR-binding site and the Fur-binding motif are shown. The nucleotide sequence between -76 and +45 relative to the transcription start site (+1) is shown (B). Putative Fur-binding motif overlapping -35 and -10 promoter elements, ribosome binding site (SD), and the N-terminal amino acids of predicted SufA protein are indicated.

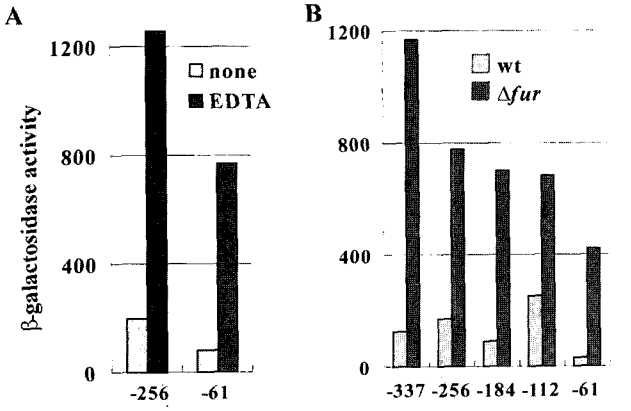

Fig. 2. Induction of *sufA-lacZ* by EDTA and *fur* mutation. (A) The *sufA-lacZ* fusion cells were grown to OD_{600}=0.2 and treated with 2 mM EDTA for 1 h. (B) A *fur* mutation allele (*fur*::Tn*5kan*) from NC442 was transduced into the various *sufA-lacZ* fusion strains by P1 transduction. Wild type and *fur* mutant cells were grown in LB at 37°C with vigorous shaking in the absence of any treatments and the basal β-galactosidase activity was measured at OD_{600}=0.5, and presented in Miller unit.

higher than that in the wild type in all the strains tested, demonstrating that the *sufA* promoter is negatively regulated by Fur. The extent of derepression remains high in the shortest *sufA-lacZ* fusion, suggesting that the Fur binding site resides between -61 and +90 nt.

Binding of Fur to sufA promoter fragment

We examined the binding of Fur to the *sufA* promoter fragment by gel mobility shift assay. DNA probes spanning from -61 to +30 nt (probe 1) and from +31 to +90 (probe 2) were used as described in Fig. 3. Fur binding

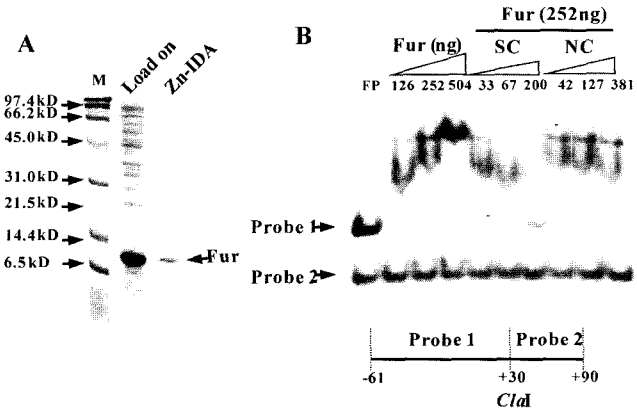

Fig. 3. Direct binding of purified Fur to *sufA* promoter fragment. (A) SDS-PAGE of purified Fur used in gel shift assay. (B) A 151 bp DNA fragment from -61 to +90 nt was amplified by PCR and labeled with [γ-^{32}P] ATP. After *Cla*I-digestion at position +30, both fragments (probe1, 2) were incubated for 20 min with increasing amounts of purified Fur. For competition experiments, the binding reaction contained 252 ng of purified Fur. Specific competitors (SC; unlabeled 151 bp *sufA* promoter DNA) were used in 33-, 67-, and 200-fold molar excess. Non-specific competitors (NC), *Hae*III-digested fragments of pGEM-3zf (+) plasmid, were used in 42-, 127-, and 381-fold molar excess over the labeled probe.

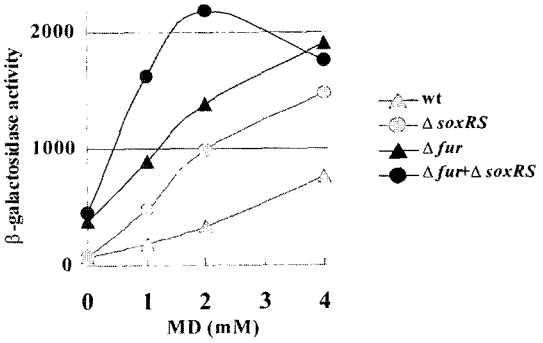

Fig. 4. Additive effect of SoxRS and Fur. The Δ*soxRS* (Δ*sox-8*::*cat*) and Δ*fur* (*fur*::Tn*5kan*) mutations were introduced into the -256 *sufA-lacZ* fusion strain. β-galactosidase activity was measured after treating varying concentrations of menadione for 1 h at early exponential phase (OD_{600}=0.2).

motif lies in probe 1. As demonstrated in Fig. 3B, Fur shifted the mobility of probe 1 only in a specific manner, confirming direct interaction of Fur with the *sufA* promoter region. To investigate whether the binding of Fur depends on the redox state, air-oxidized Fur was either untreated or pre-incubated with 1 mM DTT or 1 mM H_2O_2 before the binding reaction. Fur bound well to the *sufA* promoter fragment under all conditions tested (data not shown).

Fur regulation acts independently of the induction by oxidants

The *sufA* promoter is induced by superoxide generating agents in the absence of SoxRS (Lee and Roe, 1997).

Interestingly, we observed higher induction of *suf* genes by menadione, a superoxide generator, even in the absence of SoxRS, suggesting that SoxRS somehow represses the oxidant-induction of *sufA* (Fig. 4). It implies that SoxRS itself or the genes under SoxRS regulation could alleviate the induction of *the suf* operon by superoxide generators. When we episomally expressed SoxS on multicopy plasmid in Δ*soxRS* mutant, the induction by menadione decreased as expected (data not shown). Since the *sufA* promoter contains no SoxS-binding consensus and purified SoxS does not bind to the *sufA* promoter fragment (data not shown), the repressible effect of *soxRS* is most likely through the action of genes under *soxRS* regulation.

In the absence of Fur, even though the basal level was enhanced, the *sufA-lacZ* expression was still induced by menadione. This suggests that menadione induction of *sufA* occurs independently of Fur. We introduced double mutation of Δ*soxRS* and Δ*fur* to *sufA-lacZ* fusion strain. The double mutant was induced by menadione to a higher level than in the single mutants. From these results we conclude that regulation by Fur acts separately from the induction by oxidants or from the repressing effect by *soxRS*.

Negative effect of RpoS on the oxidant induction of sufA. Since many OxyR or SoxRS-dependent oxidant-inducible genes no longer depend on OxyR or SoxRS for oxidant induction at the stationary phase, we tested the effect of *rpoS* mutation on the oxidant induction of *sufA*. As demonstrated in Fig. 5, we found that *rpoS* also exerts a negative effect on menadione induction of *sufA*. The double mutation of *soxRS* and *rpoS* increased the induction fold

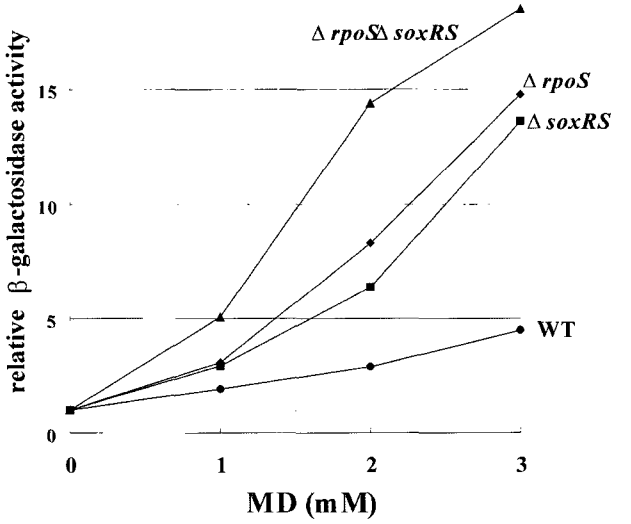

Fig. 5. Negative effect of *rpoS* on the induction of *sufA* expression. An *rpoS* (*rpoS*::Tn*10*, from KT1100) and a Δ*soxRS* mutant (Δ*sox-8*::*cat*, from BW829) alleles were transduced into the -256 *sufA-lacZ* fusion strain by P1 transduction. Cells were grown in LB broth at 37°C with vigorous shaking. Various concentrations of menadione were treated at OD_{600}=0.2 for 1 h before measuring β-galactosidase activity.

further, suggesting that the two regulatory paths act independently in *sufA* regulation.

Discussion

In this report, we demonstrated that *sufA-lacZ* expression is inducible by menadione, a superoxide generator, in the absence of SoxRS. The induction was even sensitized in the absence of SoxRS. We also presented clear evidence that *sufA* is negatively regulated by Fur, through direct binding of Fur protein to the promoter region. We also reported that the regulation by Fur and oxidant induction works independently in controlling *sufA* expression. In other words, it is highly unlikely that oxidative induction works through derepression of Fur control. From this and other works, it turns out that the *suf* operon is controlled directly by Fur and OxyR, indirectly by SoxRS and RpoS. It is not yet known what factor is responsible for inducing *sufA* in response to superoxide generators. The regulator seems negatively affected by SoxRS and RpoS, probably in an indirect manner. It is possible that antioxidant enzymes induced by SoxS and RpoS alleviate the oxidative stress. Search for the regulator, other than OxyR and Fur, that controls oxidant-responsive induction of the *suf* operon is anticipated to produce intriguing results.

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References

Althaus, E.W., C.E. Outten, K.E. Olson, H. Cao, and T.V. O'Halloran. 1999. The ferric uptake regulation (Fur) repressor is a zinc metalloprotein. *Biochemistry.* 38, 6559-6569.

Flint, D.H. 1996. *Escherichia coli* contains a protein that is homologous in function and N-terminal sequence to the protein encoded by the *nifS* gene of *Azotobacter vinelandii* and that can participate in the synthesis of the Fe-S cluster of dihydroxy-acid dehydratase. *J. Biol. Chem.* 271, 16068-16074.

Fujii, T., M. Maeda, H. Mihara, T. Kurihara, N. Esaki, and Y. Hata. 2000. Structure of a NifS homologue: X-ray structure analysis of CsdB, an *Escherichia coli* counterpart of mammalian selenocysteine lyase. *Biochemistry.* 39, 1263-1273.

Greenberg, J.T. and B. Demple. 1989. A global response induced in *Escherichia coli* by redox-cycling agents overlaps with that induced by superoxide stress. *J. Bacteriol.* 171, 3933-3939.

Greenberg, J.T., P. Monach, J.H. Chou, P.D. Josephy, and B. Demple. 1990. Positive control of a global antioxidant defense regulon activated by superoxide-generating agents in *Escherichia coli. Proc. Natl. Acad. Sci. USA.* 87, 6181-6185.

Greenberg, J.T., J.H. Chou, P. Monachch, and B. Demple. 1991. Activation of oxidative stress genes by mutations at the *soxQ/*

cfxB/marA locus of *Escherichia coli*. *J. Bacteriol.* 173, 4433-4439.

Jensen, L.T. and V.C. Culotta. 2000. Roles of *Sacharomyces cerevisiase ISA1* and *ISA2* in iron homeostatsis. *Mol. Cell. Biol.* 20, 3918-3927.

Kaut, A., H. Lange, K. Diekert, G. Kispal, and R. Lill. 2000. Isa1p is a component of the mitochondrial machinery for maturation of cellular iron-sulfur proteins and requires conserved cysteine residues for function. *J. Biol. Chem.* 275, 15955-15961.

Lee, J.H. and J.H. Roe. 1997. Isolation and characterization of paraquat-inducible promoters from *Escherichia coli*. *J. Microbiol.* 35, 277-283.

Lee, H.S., Y.S. Lee, H.S. Kim, J.Y. Choi, H.M. Hassan, and M.H. Chung. 1998. Mechanism of regulation of 8-hydroxyguanine endonuclease by oxidative stress: roles of FNR, ArcA, and Fur. *Free Radic. Biol. Med.* 24, 1193-1201.

Membrillo-Hernàndez, J., S.O. Kim, G.M. Cook, and R.K. Poole. 1997. Paraquat regulation of *hmp* (Flavohemoglobin) gene expression in *Escherichia coli* K-12 is SoxRS independent but modulated by sigma S. *J. Bacteriol.* 179, 3164-3170.

Mihara, H., T. Kurihara, T. Yoshimura, K. Soda, and N. Esaki. 1997. Cysteine sulfinate desulfinase, a NIFS-like protein of *Escherichia coli* with selenocysteine lyase and cysteine desulfurase activities. Gene cloning, purification, and characterization of a novel pyridoxal enzyme. *J. Biol. Chem.* 272, 22417-22424.

Miller, J.H. 1972. A short course in bacterial genetics: a laboratory manual and handbook for *Escherichia coli* and related bacteria. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Nachin, L., M. El Hassouni, L. Loiseau, D. Expert, and F. Barras. 2001. SoxR-dependent response to oxidative stress and virulence of *Erwinia chrysanthemi*: the key role of SufC, an orphan ABC ATPase. *Mol. Microbiol.* 39, 960-972.

Nachin, L., L. Loiseau, D. Expert, and F. Barras. 2003. SufC: an unorthodox cytoplasmic ABC/ATPase required for [Fe-S] biogenesis under oxidative stress. *EMBO J.* 22, 427-437.

Ollagnier-De Choudens, S., L. Nachin, Y. Sanakis, L. Loiseau, F. Barras, and M. Fontecave. 2003. SufA from *Erwinia chrysanthemi*: Characterization of a scaffold protein required for iron-sulfur cluster assembly. *J. Biol. Chem.* 278, 17993-18001.

Patzer, S.I., and K. Hantke. 1999. SufS is a NifS-like protein, and SufD is necessary for stability of the [2Fe-2S] FhuF protein in *Escherichia coli*. *J. Bacteriol.* 181, 3307-3309.

Pelzer, W., U. Mühlenhoff, K. Diekert, K. Siegmund, G. Kispal, and R. Lill. 2000. Mitochondrial Isa2p plays a crucial role in the maturation of cellular iron-sulfur proteins. *FEBS Lett.* 476, 134-139.

Pomposiello P.J., M.H. Bennik, and B. Demple. 2001. Genome-wide transcriptional profiling of the *Escherichia coli* responses to superoxide stress and sodium salicylate. *J. Bacteriol.* 183, 3890-3902.

Poole, R.K., M.F. Anjum, J. Membrillo-Hernàndez, S.O. Kim, M.N. Hughes, and V. Stewart. 1996. Nitric oxide, Nitrite, and Fnr regulation of *hmp* (Flavohemoglobin) gene expression in *Escherichia coli* K-12. *J. Bacteriol.* 178, 5487-5492.

Rangachari, K., C.T. Davis, J.F. Eccleston, E.M.A. Hirst, J.W. Saldanha, M. Strath, and R.J.M. Wilson. 2002. SufC hydrolyzes ATP and interacts with SufB from *Thermotoga maritima*, *FEBS Lett.* 514, 225-228.

Sambrook, J., E.F. Fritsch, and T. Maniatis, 1989. Molecular cloning: a laboratory manual. 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Silhavy, T.S., M.L. Berman, and L.W. Enquist. 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Simons, R.W., F. Houman, and N. Kleckner. 1987. Improved single and multicopy *lacZ*-based cloning vectors for protein and operon fusions. *Gene.* 53, 85-96.

Takahashi. Y., and Nakamura. M. 1999. Functional assignment of the ORF2-*iscS-iscU-iscA-hscB-hscA-fdx*-ORF3 gene cluster involved in the assembly of Fe-S clusters in *Escherichia coli*. *J. Biochem.* 126, 917-926.

Touati, D. 1983. Cloning and mapping of the manganese superoxide dismutase gene (*sodA*) of *Escherichia coli* K-12. *J. Bacteriol.* 155, 1078-1087.

Tsaneva, I.R. and B. Weiss. 1990. *soxR*, a locus governing a superoxide response regulon in *Escherichia coli* K-12. *J. Bacteriol.* 172, 4197-4205.

Walkup, L.K. and T. Kogoma. 1989. *Escherichia coli* proteins inducible by oxidative stress mediated by the superoxide radical. *J. Bacteriol.* 171, 1476-1484.

Zheng, M., X. Wang, L.J. Templeton, D.R. Smulski, R.A. Larossa, and G. Storz. 2001. DNA Microarray-mediated transcriptional profiling of the *Escherichia coli* response to hydrogen peroxide. *J. Bacteriol.* 183, 4562-4570.