Fur-mediated transcriptional and post-transcriptional regulation of FeSOD expression in *Escherichia coli*

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Fur (ferric uptake regulation protein) activates *sodB* expression, increasing expression levels by a factor of seven and *sodB* transcript stability by a factor of three. Post-transcriptional regulation of *sodB* was investigated by searching for endoribonucleases that might be involved in *sodB* mRNA degradation. The activation of *sodB* expression was significantly reduced if both the RNaseE and RNaseIII genes were mutated. This correlated with cleavage at a palindromic sequence located in the 5' untranslated region of the *sodB* transcript. An RNA-binding assay showed that Fur did not directly protect the *sodB* transcript. It was hypothesized that the persistence of Fur-mediated activation of *sodB* expression in the RNase double mutant was probably due to an effect at the transcriptional level. Therefore, it was investigated whether Fur had a direct transcriptional effect *in vitro*. Fur bound the *sodB* promoter region with low affinity, but it was not able to increase *sodB* transcription. H-NS-mediated repression of *sodB* expression, which has been shown to be Fur-dependent, was characterized. No DNA-bending region was identified in the *sodB* promoter region. H-NS did not interfere with the post-transcriptional effect of Fur. Fur-dependent H-NS and the Fur post-transcriptional effect were not additive. This suggests that Fur and H-NS effects are indirect and may be mediated by a common intermediate.

Keywords: superoxide dismutase, H-NS, RNA stability, iron

INTRODUCTION

Superoxide dismutases (SODs) are ubiquitous enzymes found in nearly all organisms and they play a major role in the multidefence system against oxidative stress (Touati, 1997). They catalyse the dismutation of the superoxide radical ($O_2^{\cdot-}$) to hydrogen peroxide (H_2O_2) and oxygen (O_2). H_2O_2 is in turn eliminated by catalases and peroxidases (Fridovich, 1997). Two cytoplasmic SODs have been identified in *Escherichia coli*, a manganese SOD (MnSOD) and an iron SOD (FeSOD), encoded by *sodA* and *sodB* respectively.

Oxidative stress is potentiated by iron because iron participates, with H_2O_2 , in the Fenton reaction, leading to the formation of the very reactive hydroxyl radical, which may react with any cellular macromolecule (Halliwell & Gutteridge, 1984; Keyer & Imlay, 1996). Iron acquisition and metabolism are therefore strictly

regulated and there is increasing evidence for co-ordination between the regulation of iron homeostasis and defence against oxidative stress (reviewed by Touati, 2000). In particular, synthesis of both the cytoplasmic SODs of *E. coli* (MnSOD and FeSOD) is controlled by the regulator of iron homeostasis, Fur (ferric uptake regulation protein) (Niederhoffer *et al.*, 1990; Tardat & Touati, 1991). In turn the expression of *fur* is regulated by two global regulator systems of oxidative stress, OxyR and SoxRS (Zheng *et al.*, 1999).

Fur, in association with Fe(II), represses the transcription of genes involved in iron uptake (Bagg & Neilands, 1987). More generally, it represses the transcription of genes involved in iron metabolism, general metabolism, stress responses, pathogenicity and various other processes (reviewed by Escolar *et al.*, 1999). The molecular mechanism of the transcriptional repression by Fur is well known. The promoters of Fur-repressed genes contain one or two target DNA sequences called iron boxes, displaying various degrees of similarity to the consensus 5'-GATAATGATAATCATTATC-3'

Abbreviations: Fur, ferric uptake regulation protein; H-NS, histone-like protein; SOD, superoxide dismutase; UTR, untranslated region.

sequence (de Lorenzo *et al.*, 1987). This sequence was initially characterized as a 19 bp palindromic sequence (Calderwood & Mekalanos, 1988) and has more recently been described as a combination of three repeats of 5'-NAT(A/T)AT-3' (Escobar *et al.*, 1998). In conditions of iron depletion, Fur associates with Fe(II) and binds as a homodimer to the iron box, rendering the promoter inaccessible to RNA polymerase, thereby inhibiting the initiation of transcription (Escobar *et al.*, 1997). All the genes involved in iron homeostasis are repressed at the transcriptional level by Fur, resulting in a correlation between iron requirements and iron uptake. The *sodA* promoter contains two iron boxes and *sodA* transcription has been shown to be repressed by Fur in an iron-dependent manner (Tardat & Touati, 1991, 1993).

A few cases of Fur-mediated activation have been reported. Fur activates the expression of *ftn* and *bfr* (iron storage), *acnA* and *fumA* (Krebs cycle) and *sodB* (FeSOD) (Andrews *et al.*, 1989; Gruer & Guest, 1994; Niederhoffer *et al.*, 1990). Positive effects of Fur have also been detected by proteome analysis after acid stress (Hall & Foster, 1996). However, nothing is known about the molecular mechanism underlying these activations. In a previous study on the regulation of *sodB* expression as a whole, we showed that Fur induced an increase in *sodB* expression by a factor of seven (Dubrac & Touati, 2000). As demonstrated for Fur-mediated repression, this activation was iron-dependent. The *cis*-acting elements involved in Fur-mediated activation were located in the 5' untranslated region (UTR) of the *sodB* transcript, encompassing a palindromic sequence followed by an AT-rich stretch. The activation appeared to be due, at least partly, to Fur-mediated *sodB* transcript stabilization. H-NS, a histone-like protein, also regulated *sodB* expression in a Fur-dependent manner, repressing *sodB* expression in the absence of Fur.

We investigated further the mechanism of Fur-mediated activation of *sodB* expression by analysing at the molecular level the transcriptional and post-transcriptional effects of Fur on *sodB* expression. Fur acted both at transcriptional and post-transcriptional level. These effects appeared to be indirect since purified Fur could not enhance *sodB* transcription nor bind the *sodB* transcript *in vitro*. The previously demonstrated effect of Fur on *sodB* mRNA stability was found to depend on an endonucleolytic cleavage and to involve RNaseIII and RNaseE. Fur-dependent effect of H-NS interfered with transcription rate and was not additional to the post-transcriptional effect of Fur.

METHODS

Bacterial strains, phages and plasmids. All strains are *E. coli* K-12 derivatives and are listed in Table 1. Basic genetic manipulations were carried out by standard procedures (Miller, 1992). $\Delta fur::kan$, $\Delta fur::cat$, *hns-1001::Tn5seq1* and $\Delta rnc::kan$ mutations were introduced by P1 transduction, selecting for antibiotic resistance 100% associated with the mutation as described previously (Compan & Touati, 1993). The $\Delta rnc::kan$ mutant was further verified as accumulating

30S RNA precursors (Babitzke *et al.*, 1993). The $\Phi(sodB-lacZ)_2$ and $\Phi(sodB-lacZ)_3$ fusions were described in a previous publication (Dubrac & Touati, 2000).

Specific strain construction. The *rne131* allele was introduced by cotransduction with *zce726::Tn10* and *rne131* mutants were selected from Tet^R transductants on the basis of their lack of growth on minimal medium containing 0.4% glycerol as sole carbon source at 44 °C (M. Dreyfus, personal communication). The *rne1 (ams1)* allele was introduced by cotransduction with *zcf229::Tn10* and the *rne1* mutants were selected from Tet^R transductants by their inability to grow at 42 °C. The $\Delta rnc::kan hns-1001::Tn5seq1 rne131$ mutant was constructed as follows. The *trpB::Tn10* mutation was introduced into a *hns-1001::Tn5seq1* mutant by P1 transduction. A P1 lysate on the resulting strain was used to cotransduce the *hns* mutation and *trpB::Tn10* into QC2922 ($\Delta rnc::kan$). Transductants were selected for tetracycline resistance and screened for *hns* mutation on the basis of their ability to grow on minimal medium containing 0.5% salicin as sole carbon source (Ohta *et al.*, 1999). Tetracycline resistance was eliminated by transduction for *trpB*⁺, selecting for tryptophan prototrophy and *hns-1001::Tn5seq1* transductants were selected as described above. The *rne131* allele was then introduced by cotransduction with *zce::Tn10* and *rne131* Tet^R transductants were selected as described above.

Media, growth conditions and β -galactosidase assays. Cells were grown in LB medium at 37 °C with shaking at 200 r.p.m. Antibiotics were added as required: ampicillin (500 μ g ml⁻¹), kanamycin (40 μ g ml⁻¹), chloramphenicol (20 μ g ml⁻¹) or tetracycline (10 μ g ml⁻¹). β -Galactosidase assays were performed as described previously (Compan & Touati, 1993).

In vitro transcription assays. DNA fragments containing the *sodB*₁₄ promoter sequence (597 bp, from -102 to +495), *hisL* promoter (388 bp, from -285 to +103) and *iucA* promoter (623 bp, from -133 to +490) were amplified by PCR using oligonucleotides *sodB5* (5'-CGGGTAATGACCCCAAAA-GAATTCGC-3') and 4218 (5'-GGTCAGCGGAGTACCCGCG-3'), *hisL5* (5'-CTGACGCGCTTCGCTGTAGC-3') and *hisL3* (5'-CCAGCACACATCGCCTG-3'), *iucA-2* (5'-GGCAAGCTTCATAATCAAACAGCTCC-3') and *iucA-3* (5'-GTGCGAAAAAGTGCGTCC-3'), respectively, from pHS1-4, chromosomal DNA and pDT10, respectively. The amplified products were purified and quantified with 33258 Hoechst reagent as described by Cesarone *et al.* (1979). Transcription reactions were performed as described by Escobar *et al.* (1997), except that the *sodB* template was used at a concentration of 0.8 nM and 0.4 U RNAP (purchased from Boehringer) was added. Samples were mixed directly with an equal volume of 7 M urea containing tracking dyes and were analysed by electrophoresis in a 6% polyacrylamide/8 M urea gel. The predicted size of the run-off *sodB* transcript thereby generated *in vitro* was 495 nt. A fragment containing the *hisL* promoter was used as a control in the transcription reaction and to normalize the quantity of product in each lane. The *hisL* template was added at a concentration of 8 nM to each transcription reaction. The lack of any effect of Fur on expression of a *hisL-lacZ* fusion was demonstrated before beginning these experiments.

DNA gel mobility shift assays. For competition assays, the *iucA* promoter fragment was prepared from pDT10 by digestion with *Bam*HI and *Hind*III, resulting in a 170 bp fragment containing two iron boxes. This fragment was eluted from the gel, dephosphorylated and end-labelled with [γ -³²P]ATP using kinase (Boehringer Mannheim). Binding assays were performed in a buffer consisting of 10 mM (BisTris)/

Table 1. Bacterial strains and plasmids

Strain or plasmid	Genotype	Source or reference
<i>E. coli</i> strains		
MG1655	F ⁻ λ^- <i>rph-1</i>	<i>E. coli</i> Genetic Stock Center
IBPC642	IBPC5321 <i>ams1</i> (T [*]) <i>zcf229::Tn10</i>	Hajnsdorf <i>et al.</i> (1994)
QC2895	WJW5 <i>rne131 zce726::Tn10</i>	M. Dreyfus, CNRS, Paris, France
SK7622	<i>thyA715 Δrnc::kan</i>	Babitzke <i>et al.</i> (1993)
BE1410	FB8 <i>bns-1001::Tn5seq1</i>	Laurent-Winter <i>et al.</i> (1997)
QC1732	F ⁻ Δ lacU169 <i>rpsL Δfur::kan</i>	Compan & Touati (1993)
QC2461	MG1655 Δ lac(IZ)	Dubrac & Touati (2000)
QC2517	MG1655 <i>recD1901::Tn10 Δfur::cat</i>	Dubrac & Touati (2000)
QC2558	QC2461 Δ fur::kan	Dubrac & Touati (2000)
QC2597	QC2461 Φ (<i>sodB-lacZ</i>) ₂	Dubrac & Touati (2000)
QC2598	QC2461 Φ (<i>sodB-lacZ</i>) ₂ Δ fur::kan	Dubrac & Touati (2000)
QC2700	QC2461 Φ (<i>sodB-lacZ</i>) ₃	Dubrac & Touati (2000)
QC2704	QC2461 Φ (<i>sodB-lacZ</i>) ₃ Δ fur::cat	Dubrac & Touati (2000)
QC2828	QC2461 Δ fur::kan(pHS1-8)	This work
QC2896	QC2461 Φ (<i>sodB-lacZ</i>) ₂ <i>rne131 zce726::Tn10</i>	This work
QC2897	QC2461 Φ (<i>sodB-lacZ</i>) ₂ <i>rne131 zce726::Tn10 Δfur::kan</i>	This work
QC2909	QC2461 Φ (<i>sodB-lacZ</i>) ₂ <i>rne131 zce726::Tn10 Δrnc::kan</i>	This work
QC2911	QC2461 Φ (<i>sodB-lacZ</i>) ₂ <i>rne131 zce726::Tn10 Δrnc::kan Δfur::cat</i>	This work
QC2922	QC2461 Φ (<i>sodB-lacZ</i>) ₂ Δ rnc::kan	This work
QC2923	QC2461 Φ (<i>sodB-lacZ</i>) ₂ Δ rnc::kan Δ fur::cat	This work
QC2970	QC2461 Φ (<i>sodB-lacZ</i>) ₂ <i>bns-1001::Tn5seq1 Δfur::cat</i> (pHS1-7)	This work
QC2975	QC2461 Φ (<i>sodB-lacZ</i>) ₂ <i>ams-1 rne-ts Δrnc::kan</i>	This work
QC2981	QC2461 Φ (<i>sodB-lacZ</i>) ₂ <i>bns-1001::Tn5seq1 rne131 zce726::Tn10 Δrnc::kan</i>	This work
QC2982	QC2461 Φ (<i>sodB-lacZ</i>) ₂ <i>bns-1001::Tn5seq1 rne131 zce726::Tn10 Δrnc::kan Δfur::cat</i>	This work
QC6014	QC2461 Φ (<i>sodB-lacZ</i>) ₂ <i>ams-1 rne-ts Δrnc::kan Δfur::cat</i>	This work
Plasmids		
pHS1-4 pHS1-7 pHS1-8	pBR322 derivatives carrying DNA fragments of various sizes (6·6, 10·5 and 3·1 kb, respectively) encompassing the <i>sodB</i> region	Sakamoto & Touati (1984)
pDT10	pUC19 derivative carrying the <i>iuc</i> region	D. Touati

borate (pH 7), 40 mM KCl, 1 mM MgCl₂, 100 μ M MnCl₂, 2 mM DTT, 5% glycerol, 5 μ g sonicated salmon-sperm DNA ml⁻¹, 100 μ g bovine serum albumin ml⁻¹ (de Lorenzo *et al.*, 1988), 10 nM purified Fur, 1·5 $\times$ 10⁻³ pmol end-labelled *iuc* promoter and the indicated amounts of unlabelled DNA. DNA competitor fragments were added to the binding mixture in a final assay volume of 10 μ l. After incubation at 37 °C for 20 min, samples were run on 4% polyacrylamide gels polymerized in 40 mM (BisTris)/borate (pH 7)/100 μ M MnCl₂. Blanks with bromophenol blue and xylene cyanol were run in the same gel to visualize the position of the front. Gels were run in the same buffer [40 mM (BisTris)/borate (pH7)/100 μ M MnCl₂] at 200 V.

RNA isolation. RNA was isolated at various times after the addition of rifampicin (150 μ g ml⁻¹) as described by Babst *et al.* (1996).

Northern blotting analysis. RNA (10–20 μ g) was subjected to electrophoresis in a 1% agarose gel. Northern blotting and hybridization (at 42 °C in 50% formamide, v/v) were performed essentially as described by Sambrook *et al.* (1989). An internal *sodB* fragment, from +306 to +495, amplified by PCR, was used as probe. The oligonucleotide 5'-ACTACC-

ATCGGCGCTACGGC-3' was used as probe to the 5S rRNA to normalize the quantity of RNA in each lane.

RNA gel mobility shift assays. A radiolabelled RNA fragment was generated by *in vitro* transcription. To obtain a large amount of transcript, the DNA matrix was fused to the T7 promoter. A 172 bp DNA fragment was generated by PCR using the following oligonucleotides: 5'-TAATACGACTC-**ACTATAGGGA***TACGCACAATAAGGC-3' for the 5' part, in which the bold sequence is the T7 promoter consensus and the asterisk shows the start site for *sodB* transcription, and 5'-CGGGATCCGCCGTAGTGATACTCGATGG-3' for the 3' part. *In vitro* transcription was performed with T7 polymerase (Promega), according to the supplier's instructions, with [α -³²P]UTP to label *de novo* synthesized RNA specifically. The product obtained was then mixed with 10 μ g yeast tRNA and 25 U RNasin (Promega) and treated with 1 U RNase-free DNaseI for 15 min at 37 °C. The RNA was precipitated with 2-propanol and resuspended in DEPC-treated water. To allow folding, the RNA was heated to 85 °C and cooled slowly to 4 °C (Alén & Sonenshein, 1999).

The radiolabelled RNA fragment (about 10000 c.p.m. per reaction) was incubated with purified Fur in 10 μ l reactions

containing 10 mM (BisTris)/borate (pH 7), 40 mM KCl, 1 mM MgCl₂, 100 µM MnCl₂ or Fe(NH₄)₂(SO₄)₂, 2 mM DTT and 5 % glycerol, according to the conditions for Fur binding established by de Lorenzo *et al.* (1988). After incubation at 37 °C for 20 min, the samples were analysed as described for DNA gel mobility shift assays (see above).

Primer extension reactions. Reverse transcription was carried out at 42 °C with AMV reverse transcriptase, according to the protocol described by Uzan *et al.* (1988), using a 25-mer oligonucleotide (5'-CGGGATCCGTAGTGATACTCGATGG-3'), 5' end-labelled, complementary to nucleotides +122 to +146 of the *sodB* gene. DNA sequencing was performed with the same primer by the dideoxy chain termination method with the Sequenase kit version 2.0 (USB) and [α -³²S]dATP (ICN).

[³H]Uridine labelling of RNA. Cells were grown to an OD₆₀₀ of about 1 in M9 medium containing 0.2 % Casamino acids at 37 °C. [³H]Uridine labelling of RNA, chase and precipitations on filters were performed as described by Mudd *et al.* (1990). The filters were then dried and the radioactivity retained determined by scintillation counting.

RESULTS

In a previous report, we showed that the expression of a *sodB-lacZ* transcriptional fusion was activated in a Fur-dependent manner. This activation was correlated with the Fur-mediated stabilization of the *sodB* transcript (Dubrac & Touati, 2000). Overall, Fur-mediated activation of *sodB* expression was significantly stronger (activation factor of seven) than Fur-mediated mRNA stabilization (factor of three to four). Thus RNA stabilization was unlikely to account entirely for the difference in *sodB* expression between *fur* and *fur*⁺ strains. We therefore questioned whether Fur could also interfere with *sodB* transcription.

Fur effect on *sodB* transcription

In a first assay we investigated whether Fur bound to the *sodB* promoter region. The capacity of the *sodB* promoter to compete *in vitro* with the *iuc* promoter was tested in gel mobility shift assays. The metal-coordinated Fur bound to the radiolabelled *iuc* promoter, as shown by de Lorenzo *et al.* (1988). The shifted Fur-*iuc* complex disappeared with a 10-times molar excess of unlabelled *iuc* promoter as competitor, whereas a 500-times molar excess of *sodB* promoter was required to obtain the same effect (Fig. 1). However, a 500-times molar excess of a non-specific DNA fragment did not act as a competitor. Considering the affinity of the *iuc* promoter for Fur, i.e. about 5 nM (Wee *et al.*, 1988), we can deduce that the *sodB* promoter has an affinity of about 250 nM for Fur. Thus, *in vitro*, Fur bound to the *sodB* promoter specifically, but with very low affinity.

We investigated the relevance *in vivo* of this low-affinity binding *in vitro* using the FURTA test (Stojiljkovic *et al.*, 1994). The *sodB* promoter carried on a high-copy-number plasmid (pUC19 derivative) did not titrate Fur, as shown by the lack of derepression of a *fhuF-lacZ* fusion (data not shown). This failure of *psodB* to titrate

Fig. 1. Competition of *psodB* with *piucA* for Fur binding analysed by gel mobility shift assay. ³²P end-labelled *iucA* promoter fragment (1.5 fmol) with no addition (lane 1) or 10 nM Fur (lanes 2–5). A *sodB* promoter fragment (190 bp), an *iucA* promoter fragment (170 bp) or a fragment of the *sodB* structural gene (190 bp) were used as competitors of Fur binding: lane 2, no competitor; 3, 15 fmol *iucA* promoter fragment; 4, 750 fmol *sodB* promoter fragment; 5, 750 fmol *sodB* gene fragment. The arrows indicate the positions of free *iucA* fragment (bottom) and of the specific *iucA* fragment/Fur complex (top).

Fur called into question the biological significance of the observed *in vitro* binding. However, because of the large number (5000 to 10000) of Fur molecules in the cell (for review see Hantke, 2001) the sensitivity of the FURTA test is not necessarily accurate for a weak promoter. Indeed despite significant Fur titration by the *iuc* promoter, we observed no significant titration of Fur by the *sodA* promoter (data not shown), although Fur binding has been clearly demonstrated *in vitro* (Tardat & Touati, 1993).

It is interesting to note the presence on the target sequence of *sodB* (Dubrac & Touati, 2000) of an array, ATAAT, preceded by a similar degenerate array. Repeats of such arrays have been shown to be involved in interaction with Fur; however, two repeats were not sufficient for stable binding (Escobar *et al.*, 1998).

We further analysed the effect of Fur on the *in vitro* transcription of *sodB*. No increase in transcription rate was observed in the presence of Fur (Fig. 2) whereas, under the same assay conditions, Fur fully repressed *iuc* transcription (data not shown). To normalize the assay, we used as an internal control of *in vitro* transcription a DNA fragment constitutively expressing a transcript similar in size to that studied; *hisL* and *thrS* were used alternately (Fig. 2). Unexpectedly, the relative efficiencies of transcription from *psodB* and *phisL* were very different. The efficiency of *in vivo* transcription from *phisL* was similar to that from *psodB*, as shown by expression of a *hisL-lacZ* transcriptional fusion (data not shown). However, *in vitro*, a 10-times molar excess of *hisL* over *sodB* DNA was required to detect a *hisL* transcript with about the same intensity (Fig. 2). Increasing the concentration of RNA polymerase by a factor of four had no effect (data not shown). However,

Fig. 2. Effect of Fur on the *in vitro* transcription of *sodB*. A 597 bp linear *sodB* template (−102 to +495) was incubated at 37 °C with purified Fur at various concentrations in a buffer containing 50 µM MnCl₂. After 5 min, *E. coli* RNA polymerase and NTPs were added. The reaction mixture was incubated for 20 min and then analysed on a denaturing polyacrylamide gel. A *hisL* DNA template (−285 to +103) was added, together with *sodB* template, to each reaction mixture to control the quality of the reaction and to normalize the quantity of product in each lane. Lanes: 1, no Fur; 2, 200 nM Fur; 3, 400 nM Fur; 4, 800 nM Fur. Positions of transcripts are indicated. The report levels of *sodB* transcript as a percentage of *hisL* are: lane 1, 158%; 2, 163%; 3, 147%; 4, 130%. Template amounts: *sodB*, 0.8 nM; *hisL*, 8 nM (ratio *sodB* DNA/*hisL* DNA, 1/10).

if the *hisL* DNA was used alone in the assay, transcription efficiency reached the expected value (data not shown). A similar result was obtained with *thrS* (data not shown). This discrepancy between the relative efficiency of transcription *in vivo* and *in vitro* indicates that RNA polymerase has a very strong affinity for the *sodB* promoter *in vitro*. This is consistent with there being a factor inhibiting *psodB* transcription present *in vivo* but absent in the *in vitro* assay.

From those results, we concluded that Fur might not directly regulate *sodB* at the transcriptional level.

Post-transcriptional Fur-mediated regulation of *sodB*

The effect of Fur on *sodB* mRNA decay may be a general RNA stabilization effect mediated by an indirect effect of Fur on some RNases. We therefore tested whether Fur stabilized the majority of *E. coli* mRNAs. The half-life of bulk mRNA was about 3 min in the parental *fur*⁺ strain and 2.7 min in the *fur* strain (data not shown). These values are similar to those previously reported for a wild-type strain (2–3 min) (Mudd *et al.*, 1990). Thus, Fur has no effect on the chemical half-life of total mRNA and the effect on the *sodB* transcript is specific.

Effect of mutations in major endoribonucleases on the expression of a *sodB-lacZ* fusion. The *cis* elements involved in Fur-mediated protection of mRNA transcript were located in the 5' UTR of the transcript, excluding a possible initial role of 3'-dependent exonucleases, the only exonucleases known to be present in *E. coli*. A single mutation in the *rnc* gene, which encodes RNaseIII, an endoribonuclease that cleaves specific double-stranded RNA structures, had no effect on expression of the fusion or on Fur-dependent activation (Fig. 3a). The *rne131* mutation in the gene of RNaseE, a single-strand-specific endoribonuclease, did not significantly affect *sodB* expression either (Fig. 3b). The *rne131* allele encodes a truncated RNaseE lacking the C-terminal part of the protein, preventing the association of RNaseE with the degradosome, but without modifying its rRNA processing activity (Lopez *et al.*, 1999). Similarly, the *rne1* mutation, which renders RNaseE catalytic activity heat-sensitive, had no effect (data not shown).

Although neither the *rne131* nor the *rnc::kan* mutations affect expression of the *sodB-lacZ* fusion, expression was significantly higher (three times) in a *fur* strain in which both RNase genes were mutated. A slightly higher level of expression was also observed in the *fur*⁺ strain (1.2 times) (see Fig. 3c). This suggests that

Fig. 3. Effects of various combinations of mutations on expression of a *sodB-lacZ* transcriptional fusion. Strains were grown in LB medium and assayed for β -galactosidase activity, expressed in units ml^{−1}, as described in Methods. The values shown are means of three experiments and individual values did not differ by more than 15% from the means. □, QC2597 (wild-type); ○, QC2598 (*fur* mutant). (a) ■, QC2922 (*rnc* mutant); ●, QC2923 (*fur rnc* mutant). (b) ■, QC2896 (*rne131* mutant); ●, QC2897 (*fur rne131* mutant). (c) ■, QC2909 (*rnc rne131* mutant); ●, QC2911 (*fur rnc rne131* mutant).

Fig. 4. Site of endonucleolytic cleavage in the *sodB* promoter region. (a) Primer extensions performed with RNA from the wild-type (QC2597) and *rnc rne131* (QC2909) strains grown to an OD_{600} of about 1 at 37 °C. RNA was extracted at various times after the addition of rifampicin. The ^{32}P -labelled oligonucleotide used for primer extension was also used for sequencing reactions in lanes G, A, T and C. (b) Primer extensions performed with RNA from the *rnc rne1* (QC2975; lane 1) and wild-type (QC2597; lane 2) strains grown at a non-permissive temperature (42 °C). These results are representative of those obtained in four independent experiments. (c) Cleavage site in the 5' UTR of the *sodB* transcript. Bold characters represent the ribosome-binding site and the start site of translation.

endoribonucleases act on the *sodB* transcript interfering with *sodB* expression. However, as stated above, RNaseIII and RNaseE are specific for different sequences or structures and this synergic effect of the double mutation is difficult to explain by classical models.

Location of an endonucleolytic cleavage involved in the Fur-mediated stabilization of the *sodB* transcript. To identify the possible site of endonucleolytic cleavage responsible for the higher level of *sodB* mRNA decay in the *fur* strain, we examined the 5' part of the *sodB* transcript by primer extension analysis in strains with mutations in genes encoding proteins with various endoribonucleolytic activities. A *sodB* transcript species shorter than the full-length transcript was identified in the wild-type strain. Cleavage occurred in the palindromic sequence located just downstream from the transcription start site (see Fig. 4). The band corresponding to the cleaved transcript was detected if primer extension was performed on RNA from a wild-type strain or from single *rnc* and *rne131* mutants (Fig. 4a and data not shown), but not if RNA from the *rnc rne131* strain was used (Fig. 4a). Cleavage was also detected in a *fur* strain but the processed transcript was more labile, suggesting greater sensitivity to further degradation (data not shown). Thus both the activity of RNaseIII and the integrity of the carboxy-terminal part of RNaseE must be disrupted to prevent this cleavage.

Different results were obtained with the *rne1* mutation

of the *rne* gene which renders the enzyme heat-sensitive. Cleavage was observed in an *rnc rne1* strain at non-permissive temperature (Fig. 4b). The expression of the *sodB-lacZ* fusion in a *fur rnc rne1* strain was the same at 30, 42 (Fig. 5b) and 37 °C (not shown), similar to expression in *fur* (Fig. 5a). This is consistent with cleavage being important for the RNA processing enhanced in a *fur* background. Noteworthy is the fact that the effect of activation by Fur of *sodB* expression in the wild-type background was temperature-dependent, being reduced at 30 °C (see Fig. 5a). This is presumably due to a temperature-dependent structural change.

The result obtained with RNA from the *rnc rne131* strain was connected with the Fur effect on a *sodB-lacZ* fusion, $\Phi(sodB-lacZ)_3$, in which the promoter region is partially deleted, the deletion including the cleavage site identified above. The $\Phi(sodB-lacZ)_3$ fusion was more strongly expressed than a fusion involving the *sodB* wild-type promoter region, $\Phi(sodB-lacZ)_2$. The increase in expression was weak in *fur*⁺ and significantly higher in *fur* mutant strains (Dubrac & Touati, 2000). The introduction of *rnc rne131* mutations did not affect the expression of the $\Phi(sodB-lacZ)_3$ fusion in either *fur*⁺ or *fur* strains (Fig. 5c). Thus deletion of the cleavage site appears to mimic effect of the *rnc rne131* mutations.

These results together strongly suggest that cleavage occurs within the palindromic sequence located at the 5' end of the *sodB* transcript and that this cleavage is

Fig. 5. Expression of *sodB-lacZ* transcriptional fusions with and without 5' UTR cleavage of the *sodB* transcript. Strains were grown in LB medium and assayed for β -galactosidase activity (expressed in units ml^{-1}) as described in Methods. The values shown are means of three experiments and individual values did not differ by more than 15% from the means. (a) $\square$, QC2597 (wild-type) at 30 °C; $\circ$, QC2598 (*fur* mutant) at 30 °C; $\blacksquare$, QC2597 at 42 °C; $\bullet$, QC2598 at 42 °C. (b) $\square$, QC2975 (*rnc rne1* mutant) at 30 °C; $\circ$, QC6014 (*rnc rne1 fur* mutant) at 30 °C; $\blacksquare$, QC2975 at 42 °C; $\bullet$, QC6014 at 42 °C. (c) Measurement of β -galactosidase activity of strains carrying the $\Phi(\text{sodB-lacZ})_3$ fusion. The palindromic region shown in Fig. 4 is truncated in $\Phi(\text{sodB-lacZ})_3$. The sequence 5'-CAATAAGGCTATTGT-3' is replaced by 5'-ATCCT-3'. This modification results in the absence of the cleavage site. $\square$, QC2700 (wild-type); $\circ$, QC2704 (*fur*); $\blacksquare$, QC2909 (*rne131 rnc*); $\bullet$, QC2911 (*fur rne131 rnc*).

Fig. 6. Effect of Fur on gel mobility shift of the *sodB* transcript. The *sodB* transcript was prepared by *in vitro* transcription using [^3H]uridine. It was then heated at 85 °C for 10 min and slowly cooled. About 10000 c.p.m. of radiolabelled mRNA was mixed with purified Fur. Lanes: 1, no Fur; 2, 100 nM Fur; 3, 300 nM Fur.

correlated with the Fur-dependent activation of *sodB* expression.

As Fur mediates stabilization of the *sodB* transcript, we investigated whether Fur protected RNA from degradation by direct binding. *sodB* RNA was heated and then cooled slowly to allow multiple possible folding. Purified Fur (at concentrations up to 300 nM) did not bind any of the refolded forms of RNA (Fig. 6). The

binding assays were performed with manganese as the Fur cofactor because this metal is more stable than iron in its reduced form in aerobiosis. An experiment performed with Fe(II) [$\text{Fe}(\text{SO}_4)_2(\text{NH}_4)_2$] as the Fur cofactor gave identical results (not shown).

H-NS-mediated repression of the *sodB* expression

H-NS reduced the level of expression of a *sodB-lacZ* transcriptional fusion if the *fur* locus was inactivated (Dubrac & Touati, 2000). To determine the level at which H-NS-mediated regulation occurred, we investigated whether H-NS modulated *sodB* mRNA degradation. As expected, given that the expression of *sodB-lacZ* was H-NS-independent in the *fur*⁺ strain, similar amounts of *sodB* transcript were detected in the wild-type and the *hns* mutant. In an *hns fur* double mutant, the half-life of the *sodB* transcript was similar to that in a single *fur* mutant (data not shown). This result strongly suggests that the observed effect of the *hns* mutation on *sodB-lacZ* expression in a *fur* background was transcriptional.

H-NS is known to bind specifically to curved DNA and thereby modulates transcription (Yamada *et al.*, 1991). A computer analysis was performed to identify possible sections of curved DNA in the *sodB* promoter region. The bending model was created with the 3D-WEDGE program (G. Micheli, Centro Acidi Nucleici CNR, Rome, Italy). A 1000 bp DNA fragment (−500 to +500 from the translation start site) was tested. The *cis* elements of the *sodB* promoter region involved in H-NS-mediated regulation are located in the 60 bp region between the transcription start site and the translation start site (Dubrac & Touati, 2000). Computer analysis showed no potential curvature in the region of the *sodB*

promoter involved in H-NS repression (data not shown). To confirm this prediction experimentally, we analysed the migration of a PCR-amplified fragment of the *sodB* promoter containing the region of interest with respect to H-NS repression. Migration in agarose at 4 and 60 °C increased and reduced, respectively, the effect of DNA bending on electrophoretic mobility (Dieckmann & Wang, 1985). We observed no differential migration at the two different temperatures (data not shown). In the light of previous studies showing a very high specificity of H-NS binding for curved DNA, these results strongly suggest that the observed repression of *sodB-lacZ* expression by H-NS did not result from binding of H-NS to the *sodB* promoter.

As H-NS repressed expression by a factor of about two, the lower level of expression in the *fur* strain may have resulted from cumulative effects: loss of RNA stabilization and transcriptional repression via H-NS. Consistent with this, it was predicted that expression of the *sodB-lacZ* fusion in a strain carrying the *fur rne131 rnc hns* mutations would reach levels about twice those in the *fur rne131 rnc* strain, and similar to those in the *fur*⁺ strain. No difference was found between the two strains, ruling out a cumulative Fur-dependent effect of RNases and H-NS mutations (data not shown).

This supported the view that the Fur H-NS-mediated effect is indirect and that Fur acts indirectly both at post-transcriptional and transcriptional levels.

DISCUSSION

Fur, which is primarily involved in the regulation of iron metabolism, is now known to regulate a large number of genes. The molecular mechanism of regulation has been studied only for Fur acting as a transcriptional repressor. There are a few reports of positive effects of Fur on gene expression, but the underlying mechanisms were never investigated. It is therefore unknown whether the activation of expression was due to a direct effect of Fur or mediated by an intermediate regulator under Fur control. Our previous study on positive Fur-mediated regulation of the *sodB* gene encoding FeSOD suggested that regulation occurred at both the post-transcriptional and transcriptional levels. The data presented here show only a weak binding of Fur to the *sodB* promoter region and no binding to the *sodB* transcript. Although these data do not authorize us to exclude the possibility that Fur activation of *sodB* expression is mediated via a direct binding of Fur on the *sodB* promoter, we favour the hypothesis of indirect regulation. (i) The target region of the Fur effect is located downstream from the transcription start site of *sodB*, a position more frequently subjected to negative than to positive transcriptional control. (ii) Although DNA-binding assays indicated some specific binding of Fur to the promoter region of *sodB*, binding efficiency was very low and possibly not significant *in vivo*. (iii) The inability of purified Fur to activate *in vitro* transcription and the much higher level of *in vitro* transcription from *psodB* than from *phisL*, despite similar levels of *in vivo*

expression, provide further evidence of an indirect effect and suggest that an inhibitory factor may be missing in the *in vitro* experiment. (iv) No binding of Fur to RNA was detected, ruling out the hypothesis that Fur acts as an RNA-binding protein. This led us to speculate that an intermediate regulator under Fur control might exist. As the basic level of *sodB* expression in the *fur* mutant is high enough, classical methods of selecting mutants for this putative regulator were not possible. An approach involving transcriptome analysis is currently being used.

One clear consequence of Fur regulation was stabilization of the *sodB* transcript. The higher level of *sodB* expression in a *fur* strain in which both major RNase genes were mutated, together with the lack of endonucleolytic cleavage of the *sodB* transcript in an *rne131 rnc* strain, suggested that both RNaseE and RNaseIII were involved in *sodB* mRNA decay. However, the process of RNA degradation is still unclear. In particular, it is not easy to account for the apparent redundancy between the actions of RNaseIII and RNaseE on the *sodB* transcript. Cleavage within the palindromic sequence at the 5' end of the transcript occurs if one of these RNases is present. There is no known example of cleavage at the same site by these two RNases and it has been clearly demonstrated that whereas RNaseIII is double-strand-specific, RNaseE can cleave in single-stranded AU-rich RNA in a 5'-dependent manner (Mackie, 1998; Nicholson, 1999). This suggests that the *sodB* transcript is degraded in several steps, including, at the late stage, events dependent on the actions of RNaseIII and RNaseE. Cleavage within the palindromic sequence may be a crucial initial event. RNaseE is an endonucleolytic enzyme involved in mRNA decay and rRNA maturation. It is the central element of the degradosome, a multienzyme complex involved in the decay of most mRNA species. The effect of RNaseE was found only in experiments carried out with the *rne131* allele, which was originally characterized in the laboratory of M. Dreyfus (Lopez *et al.*, 1999). This mutant produces an RNaseE truncated at the carboxy-terminal end, resulting in inhibition of the formation of the degradosome without the abolition of catalytic activity. Thus, the *rne131* mutation permits the further processing of rRNA and strains carrying this allele are viable, but impaired in mRNA decay. In contrast, the *rne1* mutation does not seem to inhibit degradosome formation, but at non-permissive temperature, the catalytic activity of RNaseE is abolished (Coburn *et al.*, 1999). The endonucleolytic cleavage of *sodB* mRNA was observed and no increase of *sodB-lacZ* expression was found in an *rnc rne1* double mutant at non-permissive temperature. This suggests that, rather than the catalytic activity of RNaseE, the interaction between other major components of the degradosome is important.

Fur-dependent RNA stabilization did not fully account for the activation of *sodB* expression, suggesting that an additional effect, at the transcriptional level, may be mediated by Fur. A Fur-dependent transcriptional effect mediated by H-NS was effectively observed. (i) The

effect of H-NS is not post-transcriptional because *hns* mutation in the *fur* strain did not increase *sodB* mRNA decay, whereas *sodB-lacZ* expression was increased (two to three times). (ii) The effect of H-NS is probably indirect because examination of the *sodB* promoter region revealed no putative curved DNA region, specific for recognition by H-NS. (iii) There was no cumulative effect of H-NS and RNA stabilization by inactivation of the major RNases (RNaseIII and RNaseE). This suggests a common Fur-regulated pathway leading to transcriptional and post-transcriptional effects. The numerous bands observed upon the gradual cooling of the *psodB* RNA region suggested that this RNA may take up numerous conformations. It is possible that all Fur-dependent effects, transcriptional and post-transcriptional, may be mediated by a single intermediate regulator. We speculate that the expression of the gene encoding the intermediate regulatory protein 'X' is probably negatively regulated by Fur and positively by H-NS. Only if Fur repression was relieved, could H-NS activate transcription. H-NS is known to be a transcriptional repressor, but two-dimensional protein gel analysis has shown that the expression of many genes is activated by H-NS, although the underlying mechanism is unclear. It has also been demonstrated that H-NS-mediated repression can be eliminated by inducing target-gene expression (reviewed by Atlung & Ingmer, 1997). These findings are consistent with our hypothesis: an 'X' target gene may be repressed by Fur and activated by H-NS if Fur repression is relieved.

Few proteins have been shown to bind both DNA and RNA (Shamoo *et al.*, 1993). Such binding may account for the transcriptional and post-transcriptional effects of the putative 'X' protein. We favour a model in which the 'X' protein binds to DNA in the region between the transcription and translation start sites, slowing down the progression of RNA polymerase, generating two effects: reduction of the efficiency of *sodB* transcription and formation of RNA structures more accessible to endonucleolytic cleavage. Experiments are under way to determine whether such a model of coupling between DNA transcription and RNA stabilization, consistent with several of the observations reported in this work, operates in reality.

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REFERENCES

- Alén, C. & Sonenshein, A. L. (1999). *Bacillus subtilis* aconitase is an RNA-binding protein. *Proc Natl Acad Sci USA* **96**, 10412–10417.
- Andrews, S. C., Harrison, P. M. & Guest, J. R. (1989). Cloning, sequencing, and mapping of the bacterioferritin gene (*bfr*) of *Escherichia coli*. *J Bacteriol* **171**, 3940–3947.
- Atlung, T. & Ingmer, H. (1997). H-NS: a modulator of environmentally regulated gene expression. *Mol Microbiol* **24**, 7–17.
- Babitzke, P., Granger, L., Olszewski, J. & Kushner, S. R. (1993). Analysis of mRNA decay and rRNA processing in *Escherichia coli* multiple mutants carrying a deletion in RNase III. *J Bacteriol* **175**, 229–239.
- Babst, M., Hennecke, H. & Fischer, H.-M. (1996). Two different mechanisms are involved in the heat-shock regulation of chaperonin gene expression in *Bradyrhizobium japonicum*. *J Mol Biol* **19**, 827–839.
- Bagg, A. & Neilands, J. B. (1987). Molecular mechanism of regulation of siderophore-mediated iron assimilation. *Microbiol Rev* **51**, 509–518.
- Calderwood, S. B. & Mekalanos, J. J. (1988). Confirmation of the Fur operator site by insertion of a synthetic oligonucleotide into an operon fusion plasmid. *J Bacteriol* **170**, 1015–1017.
- Cesarone, C. F., Bolognesi, C. & Santi, L. (1979). Improved microfluorometric DNA determination in biological material using 33258 Hoechst. *Anal Biochem* **100**, 188–197.
- Coburn, G. A., Miao, X., Briant, D. J. & Mackie, G. A. (1999). Reconstitution of a minimal RNA degradosome demonstrates functional coordination between a 3' exonuclease and a DEAD-box RNA helicase. *Genes and Dev* **13**, 2594–2603.
- Compan, I. & Touati, D. (1993). Interaction of six global transcription regulators in expression of manganese superoxide dismutase in *Escherichia coli* K-12. *J Bacteriol* **175**, 1687–1696.
- Dieckmann, S. & Wang, J. C. (1985). On the sequence determinants and flexibility of the kinetoplast DNA fragment with abnormal gel electrophoretic mobilities. *J Mol Biol* **186**, 1–11.
- Dubrac, S. & Touati, D. (2000). Fur positive regulation of iron superoxide dismutase in *Escherichia coli*: functional analysis of the *sodB* promoter. *J Bacteriol* **182**, 3802–3808.
- Escobar, L., de Lorenzo, V. & Perez-Martin, J. (1997). Metallo-regulation *in vitro* of the aerobactin promoter of *Escherichia coli* by the Fur (ferric uptake regulation) protein. *Mol Microbiol* **26**, 799–808.
- Escobar, L., Perez-Martin, J. & de Lorenzo, V. (1998). Binding of the Fur (Ferric Uptake Regulator) repressor of *Escherichia coli* to arrays of the GATAAT sequence. *J Mol Biol* **283**, 537–547.
- Escobar, L., Perez-Martin, J. & de Lorenzo, V. (1999). Opening the iron box: transcriptional metalloregulation by the Fur protein. *J Bacteriol* **181**, 6223–6229.
- Fridovich, I. (1997). Superoxide anion radical (O_2^-), superoxide dismutases (SODs), and related matters. *J Biol Chem* **272**, 18515–18517.
- Gruer, M. J. & Guest, J. R. (1994). Two genetically distinct and differentially regulated aconitases (AcnA and AcnB) in *Escherichia coli*. *Microbiology* **140**, 2531–2541.
- Hajnsdorf, E., Carpousis, A. J. & Régnier, P. (1994). Nucleolytic inactivation and degradation of the RNase III processed *pnp* message encoding polynucleotide phosphorylase of *Escherichia coli*. *J Mol Biol* **239**, 439–454.
- Hall, H. K. & Foster, J. W. (1996). The role of Fur in the acid tolerance response of *Salmonella typhimurium* is physiologically and genetically separable from its role in iron acquisition. *J Bacteriol* **178**, 5683–5691.
- Halliwell, B. & Gutteridge, M. C. (1984). Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem J* **219**, 1–14.

- Hantke, K. (2001). Iron and metal regulation in bacteria. *Curr Opin Microbiol* **4**, 172–177.
- Keyer, K. & Imlay, J. A. (1996). Superoxide accelerates DNA damage by elevating free-iron levels. *Proc Natl Acad Sci USA* **93**, 13635–13640.
- Laurent-Winter, C., Ngo, S., Danchin, A. & Bertin, P. (1997). Role of *Escherichia coli* histone-like nucleoid-structuring protein in bacterial metabolism and stress response. *Eur J Biochem* **244**, 767–773.
- Lopez, P. J., Marchand, I., Joyce, S. A. & Dreyfus, M. (1999). The C-terminal half of RNase E, which organizes the *Escherichia coli* degradosome, participates in mRNA degradation but not rRNA processing *in vivo*. *Mol Microbiol* **33**, 188–199.
- de Lorenzo, V., Giovannini, F., Herrero, M. & Neilands, J. B. (1988). Metal ion regulation of gene expression. *Mol Biol* **203**, 875–884.
- de Lorenzo, V., Wee, S., Herrero, M. & Neilands, J. B. (1987). Operator sequences of the aerobactin operon of plasmid ColV-K3 binding the ferric uptake regulation (*fur*) repressor. *J Bacteriol* **169**, 2624–2630.
- Mackie, G. A. (1998). Ribonuclease E is a 5'-end-dependent endonuclease. *Nature* **395**, 720–723.
- Miller, J. H. (1992). *A Short Course in Bacterial Genetics*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Mudd, E. A., Krisch, H. M. & Higgins, C. F. (1990). RNase E, an endoribonuclease, has a general role in the chemical decay of *Escherichia coli* mRNA: evidence that *rne* and *ams* are the same genetic locus. *Mol Microbiol* **4**, 2127–2135.
- Nicholson, A. W. (1999). Function, mechanism and regulation of bacterial ribonucleases. *FEMS* **23**, 371–390.
- Niederhoffer, E. C., Naranjo, C. M., Bradley, K. L. & Fee, J. A. (1990). Control of *Escherichia coli* superoxide dismutase (*sodA* and *sodB*) genes by the ferric iron uptake regulation (*Fur*) locus. *J Bacteriol* **172**, 1930–1938.
- Ohta, T., Ueguchi, C. & Mizuno, T. (1999). *rpoS* function is essential for *bgl* silencing caused by C-terminal truncated H-NS in *Escherichia coli*. *J Bacteriol* **181**, 6278–6283.
- Sakamoto, H. & Touati, D. (1984). Cloning of the iron superoxide dismutase gene (*sodB*) in *Escherichia coli* K12. *J Bacteriol* **159**, 418–420.
- Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Shamoo, Y., Tam, A., Konigsberg, W. H. & Williams, K. R. (1993). Translational repression by the bacteriophage T4 gene 32 protein involves specific recognition of an RNA pseudoknot structure. *J Mol Biol* **232**, 89–104.
- Stojiljkovic, I., Baumler, J. A. & Hantke, K. (1994). *Fur* regulon in gram-negative bacteria. *J Mol Biol* **236**, 531–545.
- Tardat, B. & Touati, D. (1991). Two global regulators repress the anaerobic expression of MnSOD in *E. coli*: *Fur* (ferric uptake regulation) and *Arc* (aerobic respiration control). *Mol Microbiol* **5**, 455–465.
- Tardat, B. & Touati, D. (1993). Iron and oxygen regulation of *Escherichia coli* MnSOD expression: competition between the global regulators *Fur* and *ArcA* for binding to DNA. *Mol Microbiol* **9**, 53–63.
- Touati, D. (1997). Superoxide dismutases in bacteria and pathogen protists. In *Oxidative Stress and the Molecular Biology of Antioxidant Defenses*, pp. 447–493. Edited by J. Scandalios. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Touati, D. (2000). Iron and oxidative stress in bacteria. *Arch Biochem Biophys* **373**, 1–6.
- Uzan, M., Favre, R. & Brody, E. (1988). A nuclease that cuts specifically in the ribosome binding site of some T4 mRNAs. *Proc Natl Acad Sci USA* **85**, 8895–8899.
- Wee, S., Neilands, J. B., Bittner, M. L., Hemming, B. C., Haymore, B. L. & Seetharam, R. (1988). Expression, isolation and properties of *Fur* (ferric uptake regulation) protein of *Escherichia coli* K12. *Biol Metals* **1**, 62–68.
- Yamada, H., Yoshida, T., Tnaka, K.-I., Sasakawa, C. & Mizuno, T. (1991). Molecular analysis of the *Escherichia coli* *bns* gene encoding a DNA binding protein, which preferentially recognizes curved DNA sequence. *Mol Gen Genet* **230**, 332–336.
- Zheng, M., Doan, B., Schneider, T. D. & Storz, G. (1999). OxyR and SoxRS regulation of *fur*. *J Bacteriol* **181**, 4639–4643.

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