Characterization of the Interaction between Fur and the Iron Transport Promoter of the Virulence Plasmid in Vibrio anguillarum

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The expression of iron transport genes fatDCBA in Vibrio anguillarum strain 775 is negatively regulated by two iron-responsive repressors, the Fur protein and the antisense RNA, RNAα. Here we report the identification of the promoter for the iron transport genes and studied the interaction between the V. anguillarum Fur protein and this promoter. The iron transport promoter was localized in a region approximately 300 base pairs upstream of fatD by both primer extension and S1 mapping analysis. High activity of the promoter was measured in response to iron depletion in the wild-type strain when a promoter-lacZ fusion was examined, whereas the promoter was constitutive in the Fur-deficient strain. Gel retardation and DNase I footprint analysis showed that Fur binds specifically to two contiguous sites comprising the promoter region and the region downstream of the transcription start site. The identified Fur binding sites showed a low degree of homology to each other as well as to the consensus sequence for the Escherichia coli Fur protein. DNase I footprints pattern suggested a sequential interaction of Fur with these two sites that renders a protection in the template strand and a hypersensitivity to the nuclease in the nontranscribed strand. The periodicity of the hypersensitive sites suggested that the promoter DNA undergoes a structural change upon binding to Fur, which might play a role in the repression of gene expression.

The marine pathogen Vibrio anguillarum strain 775 possesses a highly efficient plasmid-mediated iron uptake system that competes with the vertebrate host fish for the iron bound to high affinity iron binding proteins, such as transferrin and lactoferrin, leading to the establishment of infection (1). The pJM1 plasmid-mediated iron uptake system includes an iron scavenger, the siderophore anguibactin, and an energy-coupled iron transport system that internalizes the ferric anguibactin complex (2). The iron transport system includes an 86-kDa outer membrane receptor protein encoded by the fatA gene (3, 4), a 40-kDa cytoplasmic membrane-embedded lipoprotein that possesses periplasmic binding domains encoded by the fatB gene (5), and two 37-kDa integral membrane proteins encoded by the fatD and fatC genes (6, 7). These genes are located contiguous in the pJM1 plasmid in the order fatD, -C, -B, and -A (Fig. 1). Genetic studies utilizing transposon (Tn3::HoHo1) insertion mutagenesis have demonstrated that mutations in fatD, -C, or -B affected FatA expression, suggesting an operon organization of these genes (6, 8). A further regulatory linkage between iron transport genes and anguibactin biosynthetic genes has also been proposed since insertion mutations in the iron transport genes led to lowering or complete shutoff of anguibactin biosynthesis (8).

Expression of both, siderophore biosynthesis and iron transport genes, is regulated by positive and negative factors which function at low and high iron level, respectively. The positive regulation is mediated by the plasmid-encoded 110-kDa AngR protein whose gene lies immediately downstream of fatA and by trans acting factors encoded in a region located noncontiguous to the iron uptake region in the pJM1 plasmid (5, 8, 9, 10). The negative regulation is afforded by the chromosomally encoded Fur protein (11, 12) and by an antisense RNA (RNAα) (13). RNAα is transcribed as a counter-transcript of the fatB mRNA under the control of Fur (15). While the repression mediated by RNAα occurs at the post-transcriptional level, the Fur protein seems to regulate the expression of the iron transport genes at the transcription level. Using an RNA probe assay it has been shown that the fatB and FatA messages are no longer negatively regulated at high iron level; and therefore, their expression is constitutive when a null mutation is generated in the fur gene of the 775 strain (14).

The function of Fur in the repression of iron-regulated genes has been described in many bacteria (16); however, the interaction of Fur with its cognate operator has been characterized predominantly in the Escherichia coli plasmid aerobactin system (17–19) in which the Fur protein, in the presence of divalent metal ions, binds to a 19-bp operator sequence in the promoter region. The primary structure of the Fur protein (about 17 kDa) is highly conserved in a variety of bacteria. The comparison of predicted amino acid sequence showed that V. anguillarum Fur shares a high degree of similarity to its homolog in Vibrio species, Vibrio cholerae (94%) and Vibrio vulni ficus (92%), and a lower degree of similarity to E. coli Fur (76%) (11).

We report here the identification of the promoter that drives the expression of the polycistronic transcript of the fatDCBA genes and the interaction of the promoter with the Fur protein in vitro, to understand the regulatory mechanism of Fur in V. anguillarum. Analyses by gel retardation and DNase I footprints showed that the V. anguillarum Fur protein interacts with the promoter region in a different manner from its coun-

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1 The abbreviations used are: bp, base pair(s); kb, kilobase(s); EDDA, ethylenediamine-di-(o-hydroxyphenylacetic) acid; nt, nucleotide(s); PAGE, polyacrylamide gel electrophoresis; Bistris, 2-[bis(2-hydroxethyl)amino]-2-(hydroxymethyl)-propane-1,3-diol.
Protein-DNA Interaction Assays—*V. anguillarum* Fur, purified to homogeneity, was obtained from E. Zhelenova from the laboratory of R. Brennan. Gel mobility retardation assay was carried out with the 310-bp HindIII-EcoRI isolated from the plasmid pJHC-SC87 and labeled with [α-32P]dATP by the filling in reaction of the Klonev enzyme. The labeled DNA (24 pmol) was incubated with various concentrations of Fur protein (30 nM to 60 μM), 1 μg of poly(dI-dC), and 2 μg of bovine serum albumin in the final volume of 20 μl of reaction buffer (10 mM BisTris/boric acid (pH 7.5), 100 μM MnCl₂, 1 mM MgCl₂, and 40 mM KCl) at 37 °C for 15 min. The reaction mixture was separated by nondenaturating (5%) PAGE (in buffer (10 mM BisTris/boric acid (pH 7.5), 100 μM MnCl₂, and 40 mM KCl) at 37 °C for 20 min. The reaction mixture was exposed to a partial digestion with DNase I (Boehringer Mannheim) at 37 °C for 2 min. After stopping the reaction with 25 μg EDTA, the DNA was precipitated and analyzed by urea-PAGE (6%).

**RESULTS**

**Determination of the Transcription Start Site for fatDCBA mRNA and Identification of the Iron Transport Promoter**—To determine the transcription start of the fatDCBA mRNA, primer extension analysis was carried out with a primer (primer 1) complementary to the 5‘-end region of the fatD transcript (Fig. 2C). As shown in Fig. 2A, a number of primer extension products were observed only with RNA samples harvested under iron limitation. The pattern of the molecular species, two larger and a number of smaller bands, is identical at all three levels of iron limitation. Using molecular standards the two largest bands were estimated as 340 ± 10 nt, which maps the transcription start site(s) about 300 nt upstream of the transcriptional initiation start site of fatD. To identify the precise location of these two start sites, a further primer extension experiment was performed with a primer (Primer 2, Fig. 2A) that hybridizes at the region closer to the start site(s). The molecular weight of the two largest primer extension products was compared with a sequence obtained using the same primer (Fig. 2A) and located at 302 and 287 nt upstream of the start codon of fatD. The multiple bands detected could be generated as a result of multiple promoters and therefore multiple transcriptional start sites or processing products from larger molecules in vivo or in vitro. Alternatively they could be artifacts of the detection method, generated by the stalling of reverse transcriptase at certain stem-loops in the RNA structure, which might lead to earlier stops during the reverse transcription. To clarify the presence of multiple bands in primer extension analysis, the S1 mapping method was applied. Radiolabeled riboprobes containing either a 302-nt XmnI-EcoRI segment (obtained as a 375-nt fragment using plasmid pJHC-SC87) or a 355-nt XmnI-HindIII segment (obtained as a 609-nt fragment using plasmid pJHC-SC85) were employed in this analysis. RNA-RNA hybrids were subjected to S1 nuclease activity. Fig. 2B shows S1-mapped transcriptional start sites. As it was the case with the primer extension approach the S1 mapping method detects two major transcripts whose start sites correspond to the two major start sites identified by primer extension analysis, confirming that the RNA species present was incubated with various concentrations of Fur protein (30 nM to 60 μM), 1 μg of poly(dI-dC), and 2 μg of bovine serum albumin in the final volume of 20 μl of reaction buffer (10 mM BisTris/boric acid (pH 7.5), 100 μM MnCl₂, 1 mM MgCl₂, and 40 mM KCl) at 37 °C for 15 min. The reaction mixture was separated by nondenaturating (5%) PAGE (in buffer (10 mM BisTris/boric acid (pH 7.5), 100 μM MnCl₂, and 40 mM KCl) at 37 °C for 20 min. The reaction mixture was exposed to a partial digestion with DNase I (Boehringer Mannheim) at 37 °C for 2 min. After stopping the reaction with 25 μg EDTA, the DNA was precipitated and analyzed by urea-PAGE (6%).
here are artifacts of the detection methods. The search of the RNA structure in the fatD upstream region, by the computer program GCG, reveals at least four stem-loop structures (data not shown). When the location of the stem-loops was compared with that of the early stops detected in primer extension analysis, there was no consistency between them. The DNA sequence of the fatD upstream region is highly rich in A (78%). Therefore, it is likely that those minor products obtained with both mapping methods are generated by breathing of two strands that leads to early termination of reverse transcription or nicking by S1. It still remains to be identified whether the two major bands are both products of independent transcription initiation at different sites or if the smaller band is a specific degradation product of the larger one. According to the transcription start site mapped by the larger band (95-nt species; Fig. 2 A), we mapped a putative −10 (TAGCAT) and the −35 (CTTACA) promoter region. These sequences, however, share a low homology to the consensus sequence of the E. coli promoter. The diversity of the sequence in the iron transport promoter implies that this promoter might require a transcriptional activator that locates RNA polymerase in the promoter region and enhances its binding.

Analysis of the Iron Transport Promoter-lacZ Fusion—Since the expression of fatDCBA mRNA is regulated by the iron level, it is expected that the putative promoter, identified by primer extension, is also regulated by the iron level in the cell. To assess whether this was the case the putative promoter, called iron transport promoter, was examined using the lacZ-reporter gene expression to verify that fatDCBA genes are indeed driven from this promoter. The 1.4-kb BstEII-HinDIII fragment containing the putative promoter region (Fig. 1) was coupled transcriptionally to the lacZ-reporter gene in a pBR325 derivative plasmid, resulting in plasmid pJHC-TW95Z. The fusion construct was subsequently conjugated into the wild-type strain 775 as well as the Fur mutant strain 775MET11. Cells harboring pJHC-TW95Z were grown under various concentrations of iron. The β-galactosidase activity of the cells was measured when cells were in the early stationary growth phase, and the

**Fig. 2.** Mapping of the transcription start sites of fatDCBA mRNA. A, primer extension analysis. The total RNA preparations from strain 775 grown under various iron concentrations (lanes 1–4) were hybridized with 32P-labeled primers, which are complementary to the 5′-end region (Primer 1) and the region upstream (Primer 2) of the fatD gene (see C). The products from the subsequent reverse transcription starting from the primer were analyzed in urea-polyacrylamide gel (6%). The arrowheads indicate position of the two largest products. The supplements to the medium were: lane 1, 2 μg/ml ferric ammonium citrate; lane 2, none; lane 3, 3 μM EDDA; lane 4, 9 μM EDDA. Lane M shows DNA molecular mass marker (see “Experimental Procedures”). Lanes G, A, C, and T represent the sequence of the primer used in the respective experiment. B, S1 nuclease mapping of the 5′-end. Total RNA preparations from strain 775 grown in high iron (lanes 2, 2 μg/ml ferric ammonium citrate) or low iron (lanes 3, minimal medium alone; lane 4, 3 μM EDDA) medium were hybridized with two different riboprobes containing either the XmnI-EcoRI or XmnI-HindIII segment (see C and Fig. 1). Lanes 1, riboprobes without S1 treatment were loaded. The gel electrophoresis was carried out as described above. The size of the S1-generated products were measured by comparison with known sequence marker (not shown). C, DNA sequence of fatD 5′-end region. Only the sequence of the nontemplate strands is shown. The translational start of the fatD gene ATG and the location of the primer are indicated by bold letters. The first (+1) and second transcription start sites are indicated by small arrows.
results are presented in Fig. 3. As the diagram shows, the β-galactosidase activity of the wild-type cells measured was negligible when the cells were grown in high iron, indicating no promoter activity. However, the overall β-galactosidase activity increased significantly under iron depletion with EDDA. The maximum increase was ~200-fold at 3 μM EDDA, and the activity decreased slightly under high iron stress (up to 15 μM EDDA). Such iron-responsive activity of the promoter was not observed in the Fur mutant cells (Fig. 3). Here, the β-galactosidase activity was high under both high and low iron conditions, indicating that the promoter is constitutive in the absence of Fur in vivo. Taken together, these results strongly suggested that the promoter within the tested area is activated by iron limitation and repressed in high iron levels. Such iron regulation in a promoter region may be achieved by a complex of the repressor Fur protein with iron, as characterized in many other iron-regulated genes in Gram-negative bacteria.

**Fur Binding Assay with the Iron Transport Promoter**—The possibility that the iron regulation of the iron transport promoter is mediated by Fur-iron complex was examined in vitro. To determine the binding activity of the V. anguillarum Fur protein to the promoter, a gel retardation assay was performed under the binding reaction conditions employed for E. coli Fur binding to the aerobactin promoter (19) with few minor modifications. The detection of Fur-DNA complex was carried out in the presence of metal ion Mn²⁺, as a substitution of the natural Fur co-repressor Fe²⁺, both in the binding reaction and gel electrophoresis (nondenaturing PAGE). The 302-bp XmnI-EcoRI promoter fragment (24 μM) obtained as a 310-bp HindIII-EcoRI fragment from pJHC-SC87, was incubated with increasing concentrations of V. anguillarum Fur protein (30 nM to 60.5 μM) in the presence of 1 × 10⁻⁴-fold weight excess of nonspecific competitor DNA poly(dI-dC) in the reaction prior to the nondenaturing PAGE. As shown in Fig. 4, Fur protein specifically binds to the promoter DNA fragment. The varying amounts of Fur protein in the reaction mixture led to the detection of Fur-promoter complexes with four different gel migrations, designated as complexes a, b, c, and d in Fig. 4. At low protein concentrations, 30–120 nM monomer, only a single complex band (a) was detected, and in higher concentrations 240–960 nM two additional complex bands (b and c) appeared. While the complexes a and c seem to be formed in the same high abundance in this protein titration assay, the complex b appeared to be of lower abundance as compared with the other two complexes, a and c. From the gel retardation assay we were not able to estimate the number of Fur protein binding units on the tested DNA fragment. However, it is evident that the complexes b and c result from Fur binding at more than two different sites in the DNA fragment. The less abundant complex b implies an unstable nature of this complex, at least as assessed by the gel retardation method. This could be due to either an unstable conformation of the complex or a Fur binding site with low affinity. At protein concentrations 960 nM and higher, the protein-DNA interaction shows a plateau, indicating that all available Fur binding sites are occupied by Fur. The highest retarded complex d occurred at a 60.8 μM protein concentration. It is likely that this complex was formed as a result of nonspecific binding of Fur aggregates in the tested conditions. Although this gel retardation assay was not a detailed kinetic assay, we estimated the apparent binding constant K_app of Fur to the iucA promoter (5 nM; Ref. 28) and the sodA promoter (10–20 nM; Ref. 29).

**DNA Footprinting Analysis of Fur-Iron Transport Promoter Complex**—To locate the Fur binding sites in the 302-bp XmnI-EcoRI fragment containing the promoter region, DNase I footprinting assays were performed. The DNA fragment was radiolabeled at the 3'-end of either the template or nontemplate strand relative to the fatDCBA transcripts. The binding reaction was carried out with 1.5 nM DNA fragment and varying concentrations of the V. anguillarum Fur protein (480 nM to 60 μM) under the same binding conditions applied in the gel retardation assay. The autoradiograms of DNase I footprint patterns on both strands are shown in Fig. 5 and the summary of the Fur-DNA interaction is represented in Fig. 6. Both template and nontemplate strands showed a large interaction region with Fur that comprised about 83 and 56 nt, respectively. At low protein concentrations (480–960 nM) the template strand reveals a region of 49 nt protected from DNase I cleavage by binding to Fur (Fig. 5, lanes 8 and 9). This primary interaction site with Fur, designated as site I, is located directly in the promoter region, spanning from position +10 to –32 relative to the transcription start site, overlapping with the –10 and –35 region of the promoter. In the region between position +10 and –7 the protection was observed in only a few regions.
nucleotides. It is possible that protected nucleotides in this region are the result of the paucity of the DNase I footprints. At higher concentrations of protein (>1.9 nM) a protected region appeared in the downstream region of the transcription start site from position +17 up to +50. This secondary binding site (site II, 36 nt) is a few nucleotides smaller than the primary binding site I (42 nt), indicating that Fur binds these two sites (site II, 36 nt) is a few nucleotides smaller than the primary binding site I (42 nt), indicating that Fur binds these two sites either with different multimeric forms or in a different manner. The protection pattern in sites I and II (82 nt) was maintained in the presence of higher protein amounts (Fig. 5, lanes 1–6 and lanes 8–9).

The footprint pattern in the nontemplate strand was detected in the region spanning from position –3 up to position +49. The footprints within this region contain three areas that show hypersensitivity to DNase I cleavage. In each case, two hypersensitive nucleotides are flanked by protected nucleotides. These three hypersensitive sites appeared periodically spaced by about 15–16 nucleotides, which means a periodicity of longer than one helical turn of B-DNA. As is the case of the template strand, the footprints of the nontemplate strand revealed two Fur interaction sites (I’ and I”) that are distinguishable in affinity for Fur. The primary interaction at site I’ observed at protein concentrations 480–960 nM reveals protection and hypersensitive sites from position –6 to position +13 (Fig. 5, lanes 8 and 9) within the site I region in the opposite strand. The secondary interaction site (site II’) with two hypersensitive sites was detectable at 1.9 μM Fur in the downstream region of site I’. The site II’ is located in the complementary region of site II. The temporal and spatial coincidences of interaction sites suggested that the footprints at sites I and I’ or at site II and site II’ feature the same event with different consequences. The sensitivity of this strand was enhanced at higher concentrations of protein. The upstream region of the site I’ revealed extremely high sensitivity in a large area of about 80 nt. The hypersensitive sites appeared only in the nontemplate strand, and the main protection was observed in the template strand. These data suggested that the multimeric forms of Fur bind mainly to the template strand and change the conformation of the strand upon binding that causes a stronger exposure of the less interactive nontemplate strand to the DNase I nucleolytic attack. The enhanced sensitivity in the upstream area could be generated likewise by a conformational change of the DNA, e.g., DNA bending, presumably constrained by protein-protein interaction. Experiments to explore these possibilities are currently being carried out.

**DISCUSSION**

The regulatory function of the Fur protein in the pJM1-encoded iron uptake system has been demonstrated previously by isolating a Fur-deficient isogenic strain, a null mutant that is constitutive in both, expression of the FatB and FatA proteins, as well as in the production of dihydroxybenzoic acid, an intermediary in the biosynthesis of the siderophore anguibactin (5, 11). The aim of the present study was to characterize the repression mechanism mediated by the Fur protein in the expression of the iron transport genes, in conjunction with the identification of the promoter(s) that drive the transcription of these genes.

Primer extension analysis has identified, in accordance with S1 mapping experiments, two distinct RNA species starting from the position 302 and 287 nucleotides upstream of the fatD translation start, when the wild-type cells were grown under iron limitation. It still remains to be determined whether these two transcripts are a result of two independent transcription starts or the smaller transcript is a product of a partial degradation of the larger one at a preferential site in vivo. We presumed that, independent of the smaller RNA species, the larger species presents the transcription start site (+1), and according to this +1 site we mapped the –10 and –35 promoter region. This putative promoter, however, shows a high divergence in the –10 and –35 sequence from the promoter consensus sequence. Since the genes fatDCBA are expressed at high level under iron stress, we presumed that a transcriptional activator functioning under iron stress is involved in the stimulation on the binding of RNA polymerase to the –35 and –10 promoter region. The activity of the promoter was monitored by using a promoter fusion to the lacZ reporter gene. The β-galactosidase activity representing the promoter activity was seen in high level in response to iron depletion in the wild-type cells (Fig. 3). This corresponds to the appearance of the fatDCBA transcripts in the early stage of the iron limitation when low iron stress was applied (Fig. 2). In addition to the high activity, the promoter identified seems to possess high strength by itself, although it lacks sequence homology to the –10 and –35 consensus sequence. In the absence of positive regulators, this promoter was still able to drive the transcription at a low level.
However, no significant degree of sequence homology was found among them, except that base pair A:T in few positions of this 19-base pair operator is conserved. Our results show that *V. anguillarum* Fur interacts with a much larger DNA segment than its homolog in *E. coli*. The active polymeric state of *V. anguillarum* Fur has not been identified. However, as proposed for *E. coli* Fur (17), a dimer of the about 17-kDa (monomer) protein could interact with a 19-bp DNA segment. Therefore, it is plausible that two dimers (or a tetramer) of *V. anguillarum* Fur form a complex with each site, site I (42 nt) and site II (36 nt), as an active unit in DNA binding. To learn more about the DNA element involved in Fur binding, a search for an analog of *E. coli* Fur consensus sequence has been attempted with the sequence of the *V. anguillarum* Fur binding sites I and II. A comparison of Fur binding sequences is presented in Fig. 7. The highest similarity to the Fur consensus sequence was observed in a region of 20 nucleotides around the −10 region in site I. The other portions of the binding sites share a low similarity to the *E. coli* Fur consensus sequence. Furthermore, the characteristic of the Fur consensus sequence, a dyad symmetry, was found, although imperfect, only in the region with the highest similarity. In site I, the Fur protection was observed preferentially upstream of the −10 region, but rather weak in the −10 region. It is unlikely, therefore, that the analog to the *E. coli* consensus sequence actively participates in Fur binding in the *V. anguillarum* system. As depicted in Fig. 7, the *V. anguillarum* Fur binding sites both in the promoter region and downstream of the transcription start are highly rich in A + T. The sequence comparison among those sites shows no significant homology. However, the base pair A:T was found in a number of positions conserved in the sequence of 5′-ATnnn(A/T)T/A(nA/T)A(T)nn(T/A)nnA/T(A)n−3′. The predicted secondary structure of Fur homologs, including *V. anguillarum* and *E. coli* Fur, reveals a number of α-helices in conserved positions (30, 31).
12). Although there was no helix-turn-helix DNA binding motif found, it has been suggested that α-helices in the N-terminal region of E. coli Fur may interact with the major groove of the DNA upon sequence-specific recognition (30, 31). However, it is difficult to employ the binding mode of E. coli Fur for the V. anguillarum Fur, since the sequence-specific protein-DNA interactions through the major groove generally require a high conservation of DNA sequence, but the DNA sequences involved in the binding to V. anguillarum Fur show a high diversity without any pattern. Therefore, an alternative binding mode could be considered for the V. anguillarum Fur system that is based on low sequence specificity, e.g., recognition and binding to a specific DNA structure in the primary binding sequence.

The DNase I footprinting pattern shows different characteristics in the template (site I and II) and nontemplate strand (site I- and II'). While the template strand with sites I and II features protection by Fur, the complementary strand with site I- and II' contains at least two types of hypersensitive sites: periodic hypersensitive sites spaced by 15–16 nucleotides appeared in site I- and a cluster of hypersensitive sites in the upstream region of site I' (Fig. 6). Such hypersensitivity to DNase I is observed when protein-DNA interactions induce a conformational change of DNA, e.g., a curve in the DNA axis whereby the hypersensitive sites are at the outer face of the helical groove (32, 33). Hypersensitivity with periodicity larger than one B-helical repeat was detected when a right-handed superhelix DNA was formed by a multimeric protein, as is the case for the prokaryotic histone-like protein, H-NS (H1; Ref. 34). It is noteworthy that many proteins forming such multimeric complexes with DNA are categorized as histone-like and interact with DNA with low sequence specificity (34, 35). Interestingly, the H-NS protein binds to certain promoters with low sequence specificity and deforms the DNA structure, which results in shutting down of the expression of certain metabolic genes (33). Taken together, the binding mode of V. anguillarum Fur resembles those suggested for the prokaryotic DNA-binding protein II with histone-like properties (34, 35).

We interpret the results from DNase I footprints and gel retardation assay as follows. V. anguillarum Fur recognizes the DNA structure given by A + T-rich sequences and binds as two dimers (tetramer) primarily to the promoter region (site I), the major interaction occurring with the template strand. This step could correspond to complex a, detected in the gel retardation assay. At higher concentrations of Fur, a further tetramer binds to the secondary binding site II, the downstream region of the transcription start site. The complex formed here seems to be an intermediate and unstable (complex b) and, therefore, undergoes further conformational changes caused by protein-protein interaction between the sites I and II. The protein-protein interaction seems to accompany the formation of the superhelix in the whole Fur binding segment (80 bp), and such a distortion may enhance sensitivity to the DNase I in the outer face of the DNA helix. This complex with superhelicity (complex c) seems to render the repression mechanism by Fur in which the distortion of the DNA helix in the promoter region leads to a deteriorated positioning of the −10 and −35 region. Such a structural change is probably no longer recognizable by RNA polymerase, as shown in the mercury resistance operon when the MerR protein was bound to its operator located in the promoter in the absence of metal ion Hg2+ (36). Alternatively the presence of Fur bound in the promoter region could hinder the formation of the closed or open complex of RNA polymerase and the promoter as is the case for the H-NS mediated repression system (32).

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