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Iron Regulation of Shiga-Like Toxin Expression in *Escherichia coli* Is Mediated by the *fur* Locus

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Shiga-like toxin is an iron-regulated cytotoxin quite similar to Shiga toxin from *Shigella dysenteriae* 1. The structural genes for Shiga-like toxin in *Escherichia coli* (*sltA* and *sltB*) appear to be transcribed as an operon from a promoter upstream of *sltA*. We used a gene fusion between the promoter and proximal portion of *sltA* with the gene for bacterial alkaline phosphatase to assess the regulation of toxin expression. Growth in low-iron conditions resulted in a 13- to 16-fold increase in alkaline phosphatase activity. In the presence of a null mutation in the *fur* locus, however, alkaline phosphatase activity was constitutively high regardless of the iron concentration. These data indicate negative regulation of the *slt* operon by the *fur* gene product. We used deletion analysis of the region upstream of the gene fusion to localize the promoter of the *slt* operon and to show that a region of DNA between the −35 and −10 boxes is necessary for iron regulation of *slt* expression. In this region, there is a 21-base-pair dyad repeat that is homologous to similar dyads in the promoter regions of three other *fur*-regulated genes. This region of dyad symmetry may represent an operator binding site for the Fur protein in the presence of iron.

Certain isolates of *Escherichia coli* produce a potent protein toxin which was first detected by its cytotoxicity for Vero cells in tissue culture (14). Originally named Verotoxin, this protein has more recently been referred to as Shiga-like toxin (SLT) because of its remarkable similarity in biological activity, subunit structure, and immunological cross-reactivity to Shiga toxin from *Shigella dysenteriae* 1 (26–28). Toxins of a similar nature have been described in other species as well, including *Vibrio cholerae* (25), but the role of toxin production in the pathogenesis of infection with these organisms has not been defined.

Both Shiga toxin and SLT of *E. coli* contain a single A subunit, responsible for inhibition of protein synthesis by eucaryotic cells, and multiple copies of a B subunit, which bind the toxin to receptors on the cell surface (26, 31). The structural genes for SLT in *E. coli* are carried on temperate bacteriophage which mediate toxin conversion of the bacterial host on lysogeny (29, 39); the role of accessory genetic elements in SLT production by other species has not yet been explored. We (5a) and others (10a) have recently determined the sequence of the genes for both subunits of SLT (*sltA* and *sltB*) from *E. coli* bacteriophage H19B and 933J. We confirmed the very close structural relatedness of Shiga toxin and SLT and postulated that *sltA* and *sltB* are transcribed as an operon, because only 12 nucleotides separate the respective coding regions.

The production of both Shiga toxin (7) and SLT (27) is regulated by the iron concentration in the growth medium, with considerably more toxin made in low-iron conditions. A number of other important bacterial toxins are similarly regulated by iron, including diphtheria toxin and *Pseudomonas* exotoxin A, although the mechanism by which this regulation occurs is incompletely understood. For diphtheria toxin, there is evidence that regulation of expression by iron occurs at the level of transcription (11) and involves an operator binding site linked to the toxin structural gene (22); like SLT, the diphtheria toxin structural gene is carried on lysogenic bacteriophage. In addition, there is evidence that a protein from the bacterial host *Corynebacterium diphtheriae*, may also be necessary for repression of toxin synthesis in the presence of iron (12).

A number of different genes in *E. coli* are repressed when adequate concentrations of iron are present in the growth medium (23). These include genes responsible for the biosynthesis of iron-binding ligands (siderophores) and genes encoding inner and outer membrane proteins involved in the binding and uptake of iron-siderophore complexes. Coordinate regulation of these diverse genes occurs via a single control gene, *fur*, whose protein product acts as a negative regulator of transcription (8, 10). One model for regulation of these genes by iron involves the Fur protein as repressor and iron as corepressor, with binding of the repressor-corepressor complex to operator sites in the vicinity of the promoters of iron-regulated genes (23, 24).

In this study, we examined the regulation of expression of the *slt* operon of *E. coli* by iron and the involvement of the *fur* locus in this regulation. Our results suggest that the *slt* promoter is indeed controlled by the *fur* gene product. Comparison of the nucleotide sequence of the promoter region of the *slt* operon with that of other iron-regulated promoters in *E. coli* revealed homologous sequences that may represent the operator binding site for the Fur protein.

MATERIALS AND METHODS

Bacterial strains, plasmids, media, and reagents. The bacterial strains and plasmids used in this study are listed in Table 1. Strains CC118, CC118 (F42 *lacI3 zzf-2*::Tn*phoA* [Tn*phoA* is Tn*5* IS*50*$_L$::*phoA*]), and DHB24 were generously provided by C. Manoil, D. Boyd, and J. Beckwith; strain AB4024 was generously provided by J. B. Neilands. Bacterial cultures were grown at 37°C in LB medium (17) with shaking or on LB plates unless otherwise noted. For growth under low-iron conditions, we used either the Tris-buffered minimal medium of Simon and Tessman (38) with iron omitted (T medium) or LB medium supplemented with 0.2 mM 2,2′-dipyridyl (Sigma Chemical Co.), an iron chelator. T medium was prepared in highly purified water (Barnstead Nanopure water purification system; Sybron, Boston,

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant genotype or phenotype	Source or reference
E. coli strains		
CC118	F⁻ Δ(*ara-leu*)*7697 araD139* Δ(*lac*)*X74 phoA*Δ*20 galE galK thi rpsE rpoB argE*(Am) *recA1*	18
AB4024	F⁻ *his-4 argE thr-1 thi-1 leu-6* Δ(*lac*)*U169* Δ(*srl-recA*)*303 galK ara-14 xyl-15 mtl-1 supE44 tsx-33 fur*::Tn*5*	J. B. Neilands
DHB24	Δ(*ara-leu*)*7697 araD139* Δ(*lac*) *X74 galE galK rpsL thi malF*Δ*3 phoA*ΔPvuII *phoR zad*::Tn*10 pcnB*/F′ *lac pro lacI*^{q}	D. H. Boyd
SBC24	*fur*::Tn*5* derivative of DHB24	This study
Plasmids		
pSC2	Amp^r *sltA′*	5a
pSC105	Amp^r Kan^r *sltA′*::Tn*phoA*	This study

Mass.) and routinely supplemented with thiamine (10 μg/ml) and the L-amino acids (40 μg/ml) threonine, leucine, arginine, and histidine. The use of T medium and LB medium supplemented with dipyridyl for growth under low-iron conditions has been described previously (5, 10, 36). We verified the concentration of iron in growth medium by using 1,10-phenanthroline (Aldrich) essentially as described (15). T medium contained no measurable iron in this assay (detection limit, ≤0.5 μM), and LB medium contained approximately 10 μM. T medium was supplemented with 10 μM ferrous sulfate for growth in iron-supplemented conditions. Additional supplements for certain experiments included ampicillin (100 μg/ml) and 5-bromo-4-chloro-3-indolyl phosphate (XP; Bachem), a substrate for alkaline phosphatase (40 μg/ml).

Construction of an SltA-alkaline phosphatase gene fusion. Plasmid pSC2 contains a 1.5-kilobase-pair insert into plasmid pBR327, encoding the amino-terminal portion of SltA (including the signal sequence) and approximately 950 base pairs (bp) of additional upstream DNA (including the proposed promoter of the *slt* operon.) Tn*phoA* is a transposon vector (18) that allows the generation of gene fusions between the amino-terminal portion of a target gene and the coding sequence of bacterial alkaline phosphatase; these fusion proteins have alkaline phosphatase activity if the target genes supply a signal sequence to promote transport of the fusion protein to the periplasmic space. We followed the procedure of Manoil and Beckwith (18) to introduce the transposon Tn*phoA* from the plasmid F42 *lacI3 zzf-2*::Tn*phoA* into plasmid pSC2. Briefly, competent cells of CC118 (F42 *lacI3 zzf-2*::Tn*phoA*) were transformed with cesium chloride density gradient-purified pSC2, and the resulting transformants were plated on LB plates that contained ampicillin and kanamycin (30 μg/ml). After overnight incubation, a heavy suspension of growth from this plate was made in LB broth and spread on LB plates containing ampicillin and kanamycin (300 μg/ml); the higher concentration of kanamycin selects for transposition of Tn*phoA* (encoding kanamycin resistance) onto the high-copy-number plasmid vector. Overnight growth from each plate was suspended in LB broth, and plasmid DNA was prepared as described by Birnboim (3). Freshly competent cells of CC118 were transformed with this plasmid mixture and spread on LB plates containing ampicillin, kanamycin (30 μg/ml), and XP. Individual blue colonies appearing on this medium contained in-frame fusions of Tn*phoA* to secreted gene products on pSC2. Colonies were purified by restreaking twice on the same medium, and plasmid DNA was isolated. The presence and location of Tn*phoA* within pSC2 were confirmed by restriction mapping; plasmid pSC105 was selected for further study. The fusion joint between *sltA* and Tn*phoA* in pSC105 was subcloned into M13mp18 and M13mp19, as described by Messing (19), and the nucleotide sequence was determined by the dideoxy chain termination method of Sanger et al. (34).

Construction of deletion derivatives of plasmid pSC105. Deletion analysis was used to localize the promoter and possible iron-regulatory sites for *sltA* expression in plasmid pSC105. As shown in Fig. 1, unique restriction sites for *Eco*RV and *Sna*BI bracket an 800-bp region of insert DNA upstream of the initiation codon for the *sltA-phoA* fusion protein. Deletion 1 of plasmid pSC105 (Δ1) was constructed by digesting plasmid DNA with *Eco*RV and *Sna*BI, ligating phosphorylated *Xba*I linkers (New England BioLabs, Beverly, Mass.) to the resulting blunt-ended DNA, digesting to completion with *Xba*I, and then recircularizing the plasmid by ligation (see reference 17 for molecular biological techniques). Deletion derivatives 2 through 9 of plasmid pSC105 (Δ2 to Δ9) were constructed as follows. Plasmid DNA (approximately 30 μg) was digested to completion with *Sna*BI and then treated with BAL-31 nuclease (0.75 U; New England BioLabs) for 1 min at 30°C. The reaction was terminated by adding EGTA [ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid] to a final concentration of 20 mM and transferring to ice; DNA was recovered by phenol-chloroform extraction and ethanol precipitation. Following repair of single-stranded ends with the Klenow fragment of *E. coli* DNA polymerase I (New England BioLabs) and complete digestion with *Eco*RV, phosphorylated *Xba*I linkers were added by ligation, and the plasmids were recircularized as above. The plasmid mixture was transformed into competent cells of strain CC118, and transformants were plated on LB plates containing ampicillin, kanamycin (45 μg/ml), and XP. Plasmid DNA was isolated from 24 individual colonies, which ranged in color on the XP-containing medium from dark blue to white (depending on alkaline phosphatase activity). The sizes of the corresponding deletions were estimated by agarose and polyacrylamide gel electrophoresis (after digestion with appropriate restriction endonucleases), and the nucleotide sequence at the deletion junction was determined by DNA sequencing after subcloning into M13mp18 and M13mp19.

Assays. The fusion of Tn*phoA* to the *sltA* gene in plasmid pSC105 and its deletion derivatives allowed measurement of expression of the *slt* operon under different growth conditions by assay of alkaline phosphatase activity. Bacterial strains were grown overnight in 5 ml of T medium (with or without iron supplementation) in capped 15-ml polypropylene tubes (Corning Glass Works, Corning, N.Y.). Alkaline phosphatase activity was determined by measuring the rate

of hydrolysis of *p*-nitrophenyl phosphate (Sigma) by permeabilized cells as described (20); the alkaline phosphatase activity was expressed in units per OD_{600} of bacterial cells. To control for possible differences in plasmid copy number and nonspecific changes in transcription from plasmid-encoded genes under the different growth conditions used in our study, β-lactamase activity was determined in parallel with alkaline phosphatase activity in certain experiments. β-Lactamase activity was measured by the hydrolysis of nitrocefin as detailed by Straley and Bowmer (40); 1 U of activity (normalized to the OD_{600}) was defined as described by O'Callaghan et al. (30). Preliminary experiments demonstrated that the alkaline phosphatase and β-lactamase assays were linear over the range encountered in this study. Background levels of endogenous activity for bacterial strains not containing plasmid pSC105 or its derivatives were less than 1 U of alkaline phosphatase activity and less than 1 mU of β-lactamase activity per OD_{600} of cells.

P1 transduction. Strain AB4024 (*fur*::Tn*5*) was used as the donor for generalized transduction with P1 *vir* (37). Potential transductants were selected on LB plates containing kanamycin (30 μg/ml), and the *fur* null phenotype was confirmed by examination of outer membrane proteins from cells grown in LB broth without and with dipyridyl (see below). The *fur* null phenotype produces constitutive expression of the normally iron-regulated outer membrane proteins in the molecular weight range from 74,000 to 83,000 (13).

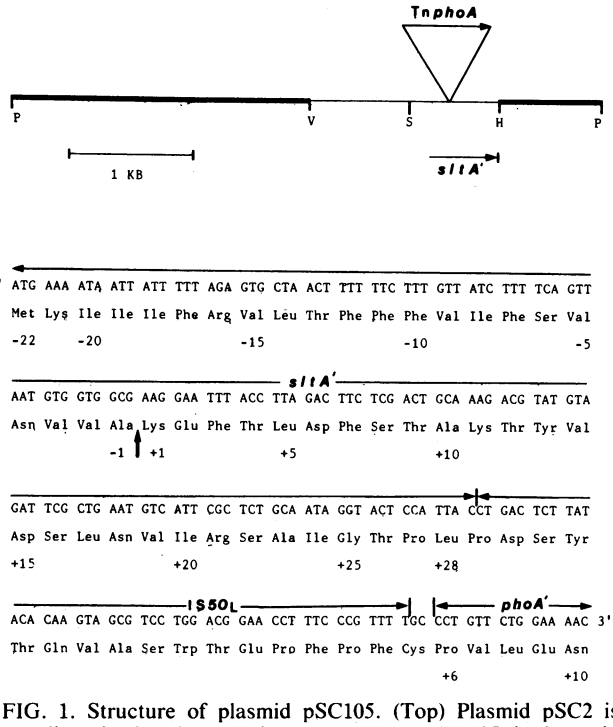


FIG. 1. Structure of plasmid pSC105. (Top) Plasmid pSC2 is shown linearized at the *Pst*I site; vector DNA (pBR327) is shown in the heavier lines, and insert DNA (from *E. coli* bacteriophage H19B) in the lighter line. The position and direction of transcription of the amino-terminal portion of *sltA* (*sltA'*, truncated at the *Hin*dIII site) are indicated below the linearized map, as is a 1-kilobase-pair (1 KB) size marker. The site of insertion of Tn*phoA* in plasmid pSC105 is shown above the map of pSC2; the arrow points from $IS50_L$ to $IS50_R$. Tn*phoA* (7,737 bp) is not shown to scale. Unique restriction sites in pSC2 are shown: P, *Pst*I; V, *Eco*RV; S, *Sna*BI; H, *Hin*dIII. (Bottom) Nucleotide and deduced amino acid sequence of the fusion protein encoded by pSC105, from the initiation codon of *sltA* across the fusion joint with $IS50_L$ and into the coding sequence of *phoA*. Negative numbers under the amino acids refer to residues in the signal sequence of SltA; positive numbers refer to residues in mature SltA (+1 to +28) or PhoA (+6 onwards). The proposed site for cleavage of the signal sequence of SltA is indicated by the vertical arrow.

TABLE 2. Alkaline phosphatase and β-lactamase assays in low-iron and iron-supplemented media

Strain	Iron added (μM)	Alkaline phosphatase activity (U)	β-lactamase activity (mU)	Induction ratio[a]
CC118(pSC105)	None	2,839	300	15.7
	10	186	309	
AB4024(pSC105)	None	897	44	1.0
	10	1,180	56	
DHB24(pSC105)	None	547	65	12.7
	10	45	68	
SBC24(pSC105)	None	576	51	1.0
	10	487	44	

[a] Ratio of alkaline phosphatase activity in low-iron compared with iron-supplemented conditions, corrected for β-lactamase activity.

Analysis of outer membrane proteins. Outer membrane protein samples were prepared from cells in late logarithmic phase in either LB broth or LB plus dipyridyl as described by Hantke (10). Equivalent amounts of outer membrane protein (approximately 20 to 30 μg) were separated through a sodium dodecyl sulfate–10% polyacrylamide gel and visualized after staining with Coomassie blue. Molecular weight standards were from Sigma.

RESULTS AND DISCUSSION

We wished to explore, at the molecular level, the mechanism by which the iron concentration in the growth medium regulates the production of SLT in *E. coli*. To allow rapid and quantitative assay of SLT expression, we used the transposon vector Tn*phoA* to create gene fusions between the promoter and proximal portions of *sltA* and the gene for bacterial alkaline phosphatase. Eight independent in-frame fusions between *sltA* and *phoA* were isolated, with the fusion joints located 150 to 550 bp downstream of the initiation codon for SltA. The fusion most proximal in SltA, carried on plasmid pSC105, was chosen for further analysis. The location of insertion of Tn*phoA* into pSC105 and the nucleotide sequence across the fusion joint are shown in Fig. 1.

Plasmid pSC105 was initially transformed into strain CC118, which carries a deletion of the chromosomal *phoA* gene. As shown in Table 2, growth of CC118(pSC105) under low-iron conditions resulted in marked induction of alkaline phosphatase activity compared with growth in the presence of iron. This induction of alkaline phosphatase activity could not be attributed to a difference in plasmid copy number or a generalized increase in transcription of plasmid-encoded genes under low-iron conditions, because β-lactamase activities in the two growth conditions were quite similar. In contrast, strain AB4024(pSC105), a mutant strain with a *fur* null phenotype, showed no significant difference in alkaline phosphatase activity between growth in low-iron and iron-supplemented conditions (Table 2).

Strains CC118 and AB4024 have many genetic differences in addition to the Tn*5* insertion at the *fur* locus. To better assess the importance of the *fur* gene product for iron regulation of SLT, we wish to construct a pair of strains isogenic except for a *fur* null mutation. For this purpose, we used strain DHB24, which carries a chromosomal deletion of *phoA*, as well as Tn*10* linked to a mutation that lowers the

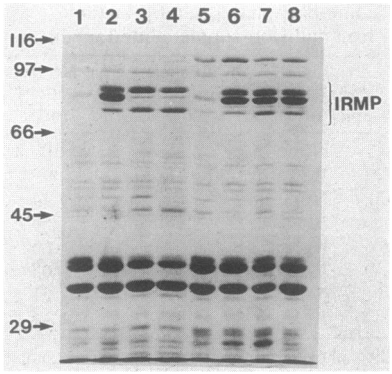
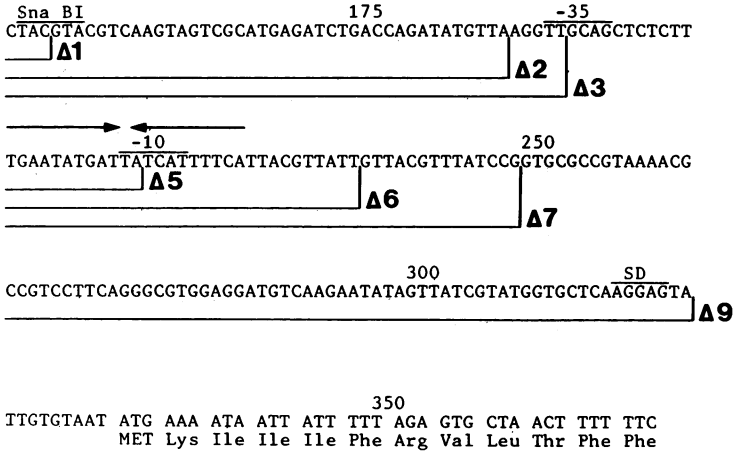


FIG. 2. Polyacrylamide gel electrophoresis of outer membrane proteins. Outer membrane protein samples (20 to 30 μg per lane) were fractionated on a sodium dodecyl sulfate–10% polyacrylamide gel. Cells were grown in LB broth without (lanes 1, 3, 5, and 7) or with (lanes 2, 4, 6, and 8) 0.2 mM dipyridyl added, and outer membrane proteins were prepared as described in the text. Strain CC118, lanes 1 and 2; AB4024, lanes 3 and 4; DHB24, lanes 5 and 6; SBC24, lanes 7 and 8. Strain AB4024 is missing one of the major iron-regulated outer membrane proteins (molecular mass, 80,500). The positions on the gel of molecular weight (10^3) standards are indicated on the left; the iron-regulated outer membrane proteins (IRMP) have apparent molecular masses of 74,000 to 83,000 daltons.

copy number of plasmid pBR322 and its derivatives (*pcnB* [16]); this latter mutation was important because plasmid pSC105 was unstable at high copy number in *fur* mutant strains (perhaps because of a deleterious effect of high-level expression of the SltA-PhoA fusion protein). Generalized transduction with P1 was used to transfer the *fur* null mutation from strain AB4024 to DHB24. The resulting strain, SBC24, constitutively expressed the iron-regulated outer membrane proteins (Fig. 2), as expected for the *fur* null phenotype. Assay of this pair of isogenic strains confirmed that the *fur* null transductant, SBC24, had constitutively high alkaline phosphatase activity, equal to the fully induced level in strain DHB24 (Table 2). This effect cannot be explained by differences in the concentration of intracellular iron between the two strains, since the *fur* null mutant constitutively expresses the iron uptake systems of *E. coli* and should have intracellular iron concentrations as high as or higher than the parent strain, DHB24. These results indicate that regulation of the *slt* operon in *E. coli* by iron depends on the chromosomal *fur* locus.

We next used deletion analysis of plasmid pSC105 to localize the promoter of the *slt* operon and to investigate whether iron regulation of *slt* expression depends on a particular region of DNA upstream of the initiation codon for *sltA*. A series of deletion derivatives of pSC105 were constructed, with each deletional endpoint brought back to an identical upstream restriction site (*Eco*RV) through an *Xba*I linker. Deletion of the approximately 800 bp between the *Eco*RV and *Sna*BI sites in pSC105 had essentially no effect on the level of expression of alkaline phosphatase activity or on regulation by iron when assayed in strain CC118 (Δ1, Fig. 3). Similarly, pSC105 (Δ2) showed parental levels of alkaline phosphatase activity and normal iron regulation at both high (strain CC118) and low copy number (strain DHB24), while in the *fur* null host (SBC24) alkaline phosphatase activity was constitutively high. A plasmid carrying deletion 3, which removed only an additional 5 bp beyond the endpoint of deletion 2, showed 5- to 10-fold less alkaline phosphatase activity at high copy number (in CC118) and 20-fold less activity at lower copy number (in DHB24). This deletion removed the first two nucleotides of the previously proposed −35 box of the *slt* promoter (5a) and thus provides additional evidence for the correct localization of the *slt* promoter.

Iron regulation of alkaline phosphatase activity for



AP Activity of pSC105 Deletions in Three Bacterial Strains, Grown in Low Iron/High Iron T Medium

	CC118	DHB24	SBC24
	3,084/534	ND	ND
Δ2	2,797/281	457/54	595/369
Δ3	561/28	21/3	52/30
Δ5	35/16	4/2	3/3
Δ6	46/26	ND	ND
Δ7	37/22	ND	ND
Δ9	5/3	ND	ND

FIG. 3. Deletion analysis of plasmid pSC105. (Left) Nucleotide sequence is shown from the *Sna*BI site to residue 12 in the signal sequence of *sltA*; nucleotide numbering above the sequence corresponds to that presented previously for the *slt* operon of *E. coli* (5a). Previously proposed −35 and −10 boxes, as well as the Shine-Dalgarno sequence (SD), are shown. The endpoints of individual deletions (Δ1 to Δ9) are shown below the corresponding nucleotide sequence; each deletion was brought back to an identical upstream sequence (5′-GATCTCTAGAG-3′). Horizontal arrows indicate a 21-bp interrupted dyad repeat overlapping the −10 box. (Right). Alkaline phosphatase (AP) activity of individual pSC105 deletions is indicated on the corresponding lines; activities were determined in low-iron T medium/iron-supplemented T medium. ND, Not done.

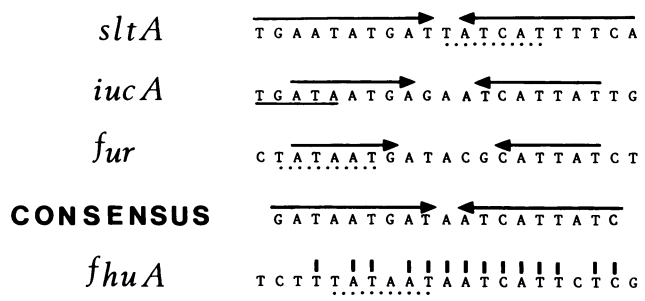


FIG. 4. Nucleotide homology between the promoter regions of several iron-regulated genes in *E. coli*. Arrows above the sequences refer to areas of dyad symmetry. The major promoter of *iucA* (P1) was used for this analysis (2). A consensus sequence was derived as a two out of three (or better) match between *sltA*, *iucA*, and *fur* that maintained dyad symmetry. Proposed −10 boxes are indicated by dotted lines under the nucleotides and −35 boxes by solid lines. The vertical dashes over the *fhuA* sequence indicate bases identical to the proposed consensus sequence.

pSC105 (Δ3) was normal despite the reduction in activity overall. Deletions 5, 6, and 7 of pSC105, which removed most or all of the *slt* promoter, showed roughly a 100-fold reduction in alkaline phosphatase activity, as well as loss of iron regulation; residual alkaline phosphatase activity from these deletion derivatives probably depends on a weak cryptic promoter upstream in vector DNA that is best detected under high-copy conditions (strain CC118). Deletion 9, which resulted in removal of the Shine-Dalgarno sequence of *sltA*, lowered alkaline phosphatase activity even further. A comparison of the iron regulation of alkaline phosphatase activities for plasmids pSC105 (Δ3) and pSC105 (Δ5) indicates that the region of DNA between these deletional endpoints is important in the control of *slt* expression by iron.

We previously noted a 21-bp dyad repeat in the vicinity of the promoter of the *slt* operon (5a), and this dyad was located in the region identified above as important in iron regulation (Fig. 3). Since bacterial operator sequences frequently show such twofold symmetry in the vicinity of a promoter (32), we considered the possibility that this region of DNA was involved in the transcriptional regulation of the *slt* operon by *fur* and iron. Accordingly, we wished to compare the promoter region of the *slt* operon with those of other genes in *E. coli* which are regulated by *fur*. Nucleotide sequence information is presently available for two such genes (*iucA* and *fhuA*) as well as for *fur* itself (2, 6, 35). If the *fur* locus is autoregulatory, as known for other repressor proteins (9), the promoter region of *fur* may share a homologous operator binding site with other *fur*-regulated promoters. With the homology search program of Intelligenetics (Intellicorp, Palo Alto, Calif.) and focusing on the region upstream of *sltA* that contains the 21-bp dyad repeat, it became apparent that the genes for *sltA*, *iucA*, and *fur* all had a region of homologous dyad symmetry in the vicinity of their promoters (Fig. 4). A consensus sequence was tentatively derived from this homology that was itself a perfect 19-bp hyphenated dyad repeat.

The putative promoter region of *fhuA* also had homology with the right-hand side of our proposed consensus sequence, but the homologous region did not exhibit dyad symmetry. Expression of *fhuA* is regulated by the iron concentration of the medium, but repression of *fhuA* at high iron concentrations is much less striking than for several other iron-regulated genes (10), including *iucA* (5) and *sltA* (this study), and the kinetics of repression and derepression are also quite different (13). Further investigation will be needed to determine whether the noted variation from our proposed consensus sequence explains the observed differences in regulation of *fhuA* by iron.

The existence of a homologous dyad repeat in these iron-regulated promoters suggests that this sequence may be involved in iron regulation and could represent the operator site recognized by the Fur-iron complex. Further experiments to test directly the validity of this model are under way.

Although the structural genes of the *slt* operon in *E. coli* are carried by temperate bacteriophage, regulation of this operon by iron depends on a separate chromosomal locus of the host bacterium, the *fur* gene. Accessory genetic elements (plasmids, transposons, and bacteriophages) play an important role in the genetics of many bacterial virulence determinants (1). Since pathogenesis often requires the interaction of several different factors, the coordinate regulation of virulence genes, including those on accessory genetic elements, frequently depends on a chromosomal regulatory locus responsive to environmental stimuli. In *V. cholerae*, for example, the chromosomal *toxR* gene encodes a positive regulator that controls production of cholera toxin, outer membrane proteins, and a pilus colonization factor (*tcpA*) (21). For enteric pathogens, the expression of iron uptake systems is essential to colonization and growth in animal tissues (4). The negative transcriptional regulation of both siderophore-based iron uptake systems and SLT production by *fur* is therefore another example of the coordinate expression of bacterial virulence factors (33, 40–42). Regulatory proteins such as Fur play an important role in pathogenesis through their response to environmental signals that pathogenic organisms may use to recognize their entry into the host. The exceedingly low availability of free iron in mammalian tissues is one such environmental signal that can trigger the coordinate expression of bacterial virulence determinants.

ACKNOWLEDGMENTS

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ADDENDUM

After submission of this paper, DeLorenzo et al. (J. Bacteriol. **169**:2624–2630, 1987) reported evidence for a Fur-binding site(s) upstream of the *iucA* gene of *E. coli* and derived a Fur-binding consensus sequence identical to that proposed in this paper.

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