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Nucleotide Sequence and Promoter Mapping of the *Escherichia coli* Shiga-Like Toxin Operon of Bacteriophage H-19B

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We determined the nucleotide sequence of the Shiga-like toxin-1 (SLT-1) genes carried by the toxin-converting bacteriophage H-19B. Two open reading frames were identified; these were separated by 12 base pairs and encoded proteins of 315 (A subunit) and 89 (B subunit) amino acids. The predicted protein subunits had N-terminal hydrophobic signal sequences of 22 and 20 amino acids, respectively. The predicted amino acid sequence of the B subunit was identical to that of the B subunit of Shiga toxin. The A chain of ricin was found to be significantly related to the predicted A1 fragment of the SLT-1 A subunit. S1 nuclease protection experiments showed that the two cistrons formed a single transcriptional unit, with the A subunit being proximal to the promoter. A probable promoter was identified by primer extension, and transcription was found to increase dramatically under conditions of iron starvation. A 21-base-pair sequence with dyad symmetry was found in the region of the SLT-1 −10 sequence, which was found to be 68% homologous to a region of dyad symmetry found in the −35 region of the promoter of the *iucA* gene on plasmid ColV-K30, which specifies the 74,000-dalton ferric-aerobactin receptor protein. Betley et al. (M. Betley, V. Miller, and J. Mekalanos, Annu. Rev. Microbiol. 40:577–605, 1986) have recently summarized evidence suggesting that the *slt* operon is under the control of the *fur* regulatory system. The area of dyad symmetry found in both promoters may represent a regulatory site. A *rho*-independent terminator sequence was found 230 base pairs downstream from the B cistron stop codon.

Shigella dysenteriae 1 produces a toxin which is cytotoxic to eucaryotic cell lines (8). Binding to the glycolipid globotriaosylceramide (Gb_3) membrane receptor is mediated by a pentamer of 7-kilodalton (kDa) B subunits, while the 31-kDa A subunit, after proteolytic nicking and reduction, inhibits protein synthesis by catalytic inactivation of the 60S ribosomal subunit (8, 20, 34, 35). O'Brien and LaVeck (30, 31) have shown that some *Escherichia coli* strains produce large amounts of a cytotoxin which appeared very similar to Shiga toxin with respect to its subunit structure and mechanism of action. It was completely neutralized by antiserum raised against Shiga toxin and was named Shiga-like toxin 1 (SLT-1) (28, 42). Recently, Strockbine et al. (42) have characterized a second cytotoxin, also produced by *E. coli*, which is related to SLT-1 at the DNA sequence level but is not neutralized by antiserum raised against Shiga toxin or SLT-1; it has been designated Shiga-like toxin 2. Both toxins have become the subject of intense study because Shiga-like toxin (SLT)-producing *E. coli* have been shown to be associated with diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (21, 33). Moreover, there have been reports (29, 32) that many pathogens, including *Vibrio parahaemolyticus*, *Vibrio cholerae*, and *Salmonella typhimurium*, produce similar toxins.

Recently, the structural genes for SLT-1 have been cloned from the toxin-converting bacteriophages H-19B and 933J (19, 28, 41). Betley et al. (1) have also reported that SLT-1 synthesis is negatively regulated by the iron-responsive *fur* gene product. In this report we present the nucleotide sequence of the A- and B-subunit cistrons of SLT-1 cloned from bacteriophage H-19B. The predicted amino acid sequence for the B subunit is identical to that recently reported for the B subunit of Shiga toxin. Results of the study of transcripts suggest that both cistrons are transcribed as a single unit from a promoter that was identified by primer extension.

MATERIALS AND METHODS

Strains and media. The *E. coli* lysogen C600 (H-19B) and strains carrying various fragments of the toxin-encoding region cloned in pUC18 or pUC19 have been described previously (19). The recombinant plasmid pJLB28 carries a *Bgl*II-*Bal*I fragment of bacteriophage H-19B inserted in pUC19 and specifies production of biologically active SLT. The size of the insert is now thought to be about 2.0 kilobase pairs (kbp) rather than 1.7 kbp as reported previously (19). pJLB26 carries a *Bal*I-*Hin*dIII fragment inserted in pUC18. It specifies production of the SLT B subunit alone (18). The host for the recombinant plasmids was *E. coli* TB1 (19). *E. coli* HB101 *galK2* containing the plasmid pK01 or pKG1800 was used for transcriptional fusion experiments (26). M13 mp18 and mp19 were obtained from Bethesda Research Laboratories (Gaithersburg, Md.). Strains were grown in L-broth or on L-agar supplemented as necessary with carbenicillin 50 (μg/ml) and 5-bromo-4-chloro-3-indolyl-β-galactopyranoside (50 μg/ml; Boehringer Mannheim Biochemicals, Canada). Plasmid pK01 and pKG1800 constructions were screened for production of galactokinase by using MacConkey agar base or M9 Casamino Acids (Difco Laboratories, Detroit, Mich.) agar supplemented with galactose (Sigma Chemical Co., St. Louis, Mo.).

M13 phages were propagated, and DNA was purified as described by Messing and Vieira (27). For blunt-end ligations, linearized M13 mp18 or mp19 replicative intermediates were dephosphorylated with calf intestinal alkaline phosphatase. Ligations were performed as described previously (19). One deletion was prepared by *Bal* 31 digestion followed by subcloning as described previously (25). Nested

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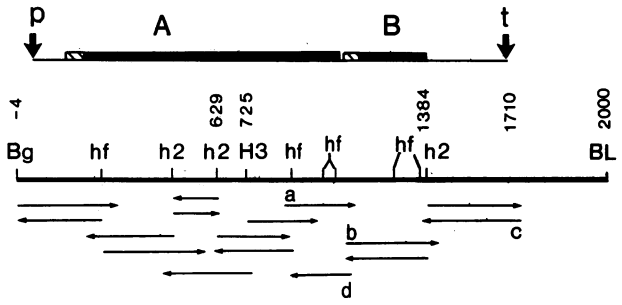

FIG. 1. Sequencing strategy. Most of the sequence was determined by subcloning restriction fragments in M13 mp18 and mp19. Some areas were determined by sequencing deletants produced in vitro by *Bal* 31 digestion (b) or sequential digestion with exonuclease III and S1 nuclease (a and c). Sequence d was determined by using a synthetic oligonucleotide primer. The *Bgl*II site is 5 bp to the left of the start of the sequence shown in Fig. 2 and is therefore designated −4. A diagrammatic model of the operon is shown above the restriction map. Transcription (thin line) started at p and ended at t. The A- and B-subunit coding sequences are shown as hatched (signal peptide) and closed (mature peptide) bars, respectively. Abbreviations: Bg, *Bgl*II; hf, *Hin*fI; h2, *Hin*cII; H3, *Hin*dIII; BL, *Bal*I.

deletions were prepared by sequential digestion with exonuclease III (Bethesda Research Laboratories) and S1 nuclease (Pharmacia Fine Chemicals, Piscataway, N.J.), as recommended by the suppliers. The mixture was phenol extracted, and the fragments were recovered by ethanol precipitation. They were then ligated with dephosphorylated M13 mp19, which had been cleaved with *Hin*cII and one other appropriate enzyme.

DNA sequencing. DNA sequencing was performed by the dideoxy chain-termination method described by Sanger et al. (37) by using [^{35}S]dATP (Amersham Corp., Arlington Heights, Ill.) as the labeled nucleotide. Dideoxynucleotides were obtained from Bethesda Research Laboratories, and deoxynucleotides were obtained from Pharmacia. The complete sequence of both strands was determined for the areas reported.

RNA was extracted from early-log-phase cultures, as described by Von Gabain et al. (45). RNA was treated with RNase-free DNaseI (Boehringer Mannheim) for 10 min at 37°C. The reaction was phenol extracted, and the RNA was recovered by ethanol precipitation prior to hybridization. Labeled single-stranded DNA was prepared by growth of M13 in JM101 in the presence of $^{32}P_i$. Hybridizations were carried out with 100 μg of DNaseI-digested total cellular RNA–80% formamide–400 mM NaCl at 53°C for 3 h. S1 nuclease digestions were carried out at 37°C for 60 min. Protected DNA species were fractionated by gel electrophoresis on a 5% acrylamide gel under denaturing conditions. End-labeled pBR322 *Hpa*II-digested DNA and phage lambda DNA digested with *Eco*RI-*Hin*dIII were used as molecular weight standards (36).

Primer extension analysis of transcripts was performed as described by Fouser and Friesen (13). A total of 2 pmol of ^{32}P 5′-end-labeled primer was mixed with 60 μg of total cellular RNA, coprecipitated, and suspended in 7.5 μl of 50 mM Tris (pH 8.3)–40 mM NaCl–0.5 mM EDTA. After heating to 65°C the primer was annealed at 45°C for 1 h. A total of 3.5 μl of mixture R (70 μM each of dATP, dGTP, dCTP, and dTTP; 30 mM $MgCl_2$, 3 mM dithiothreitol; 0.6 μg of actinomycin D per ml), and 20 U of reverse transcriptase (Life Sciences, Inc., St. Petersburg, Fla.) were added. After incubation at 42°C for 1 h the reaction was terminated by adding 5 μl of sequencing dye and heating in a boiling water bath. A total of 5 μl was run on a 5% acrylamide sequencing gel beside a [^{35}S]dATP dideoxy sequencing ladder produced by extension of the same primer annealed to the antisense strand carried in M13. Oligonucleotides were synthesized by Jacques Archambault on an automatic DNA synthesizer (Applied Biosystems). To identify the major promoter a 19-mer complementary to positions 260 to 278 of the antisense strand was used.

For iron regulation studies, C600 (H-19B) was grown in L-broth with aeration to an optical density at 600 nM of 0.15. At this point, the culture was divided into three portions. α,α′-Dipyridyl (200 μM) was added to one culture, $FeSO_4$ (50 μM) was added to a second culture, and the remainder was kept as a control culture. All three were incubated with aeration for 180 min, at which time RNA was extracted and serial dilutions of the supernatant were tested for toxin by the Vero cell assay as described previously (19).

Fusion constructions. The 730-bp *Bgl*II-*Hin*dIII fragment carrying the putative major promoter prepared as an *Eco*RI-*Hin*dIII fragment from pJLB28 and was cloned between the *Eco*RI and *Hin*dIII sites of pK01 (26). The 659-bp *Hin*dIII-*Hin*cII fragment containing a putative B-subunit promoter was cloned between the *Hin*dIII and *Sma*I sites of pK01 (26). The *Hin*cII-*Bal*I fragment was cloned in the *Sma*I site of pKG1800.

Computer analysis of DNA and protein sequences was performed by using the University of Wisconsin UWGCG sequence analysis package, as well as the programs FAST P and ALIGN from the National Biomedical Research Foundation (7). Conserved amino acid substitutions were defined as suggested in the UWGCG program SIMPLIFY: neutral or weakly hydrophobic (P, A, G, S, T); hydrophilic, acid amine (Q, N, E, D, B, Z); hydrophilic, basic (H, K, R); hydrophobic (L, I, V, M); hydrophobic aromatic (F, Y, W); cross-link forming (C).

RESULTS

The sequencing strategy used in this study is shown in Fig. 1. Restriction fragments were used as indicated in Fig. 1 for sequencing the A-subunit cistron. Other areas were sequenced by using restriction fragments and deletions constructed with *Bal* 31 nuclease or sequential digestion with exonuclease III and S1 nuclease. These sequences are indicated in Fig. 1 as b, a, and c, respectively. One additional region was sequenced by using a synthetic oligonucleotide primer (Fig. 1, sequence d). The sequence that was determined (Fig. 2) extended 1,710 bp from the first base pair after the 6-bp *Bgl*II recognition site.

Two open reading frames encoding proteins of 315 (5′ proximal) and 89 amino acids, designated as A and B cistrons, respectively, were identified. Both cistrons are preceded by Shine-Dalgarno sequences (40). In the case of the A-subunit cistron, the A subunit of the Shine-Dalgarno sequence was separated from the ATG codon by 12 bp. Kyte-Doolittle hydropathy plots (data not shown) demonstrated hydrophobic N-terminal sequences that were found to be typical of signal peptides in that both had an N-terminal positively charged sequence, a central hydrophobic sequence, and a C-terminal six-residue neutral sequence. The −1, −3 rule described by von Heijne (46, 47) was used to predict the signal peptide cleavage sites, which are indicated in Fig. 2.

The amino acid sequence predicted for the processed B subunit was identical to that of the B subunit of Shiga toxin, which was recently reported by Seidah et al. (39). The

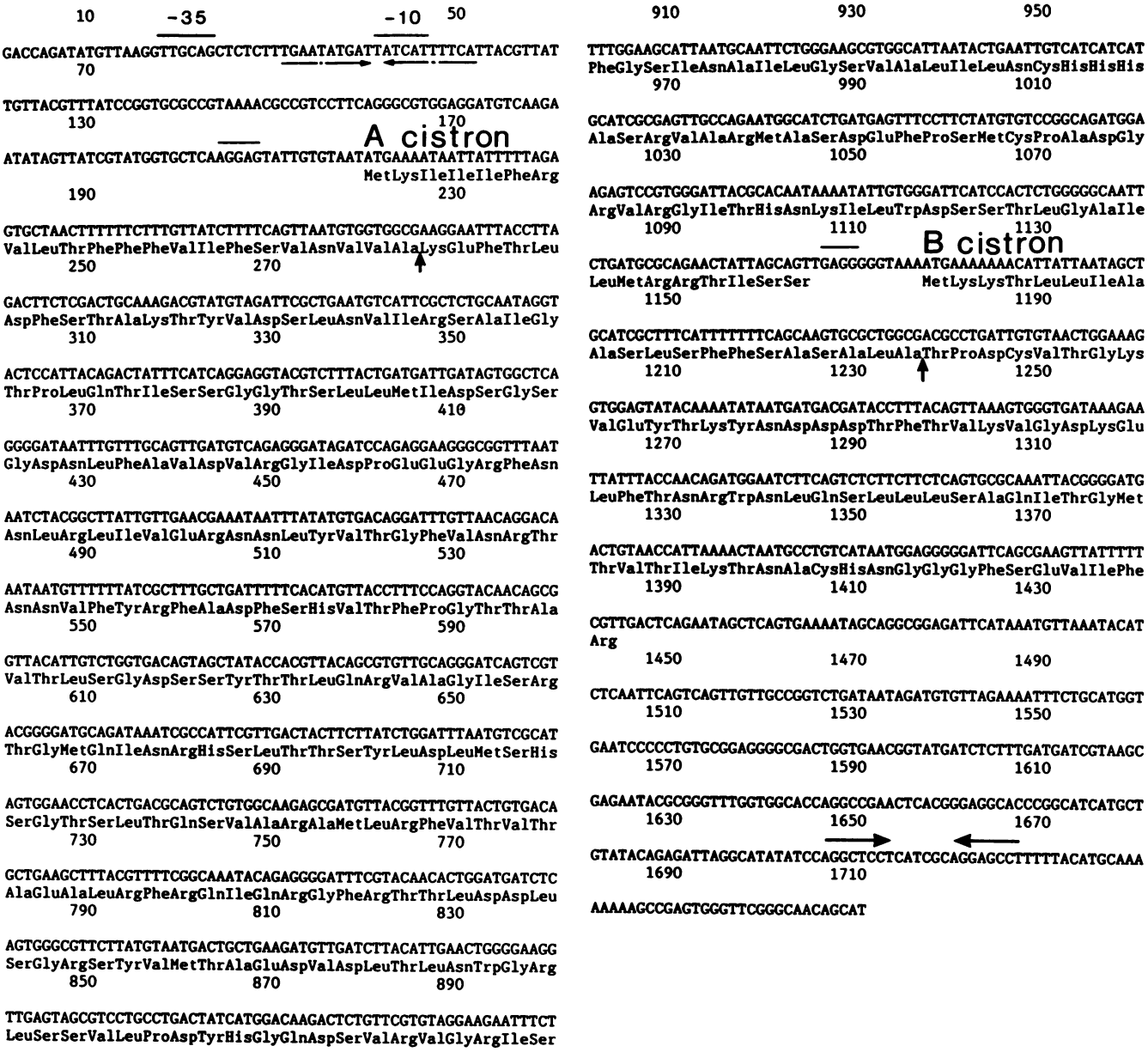

FIG. 2. Nucleotide sequence of the SLT-1 operon extending from the *Bgl*II site 1,710 bp downstream. Proposed signal peptidase cleavage sites are indicated by vertical arrows. Proposed Shine-Delgarno sequences are indicated by the lines above the sequence. The dyad symmetry overlapping the −10 sequence is indicated by horizontal arrows beneath the sequence. The stem of the putative *rho*-independent terminator is indicated by opposing arrows over the sequence. The sequence is numbered from the first base pair to the right of the 6-bp *Bgl*II recognition sequence.

calculated molecular weight was 7,691. The calculated molecular weight of the predicted amino acid sequence of the processed A subunit was 32,217. The number of residues of threonine, valine, leucine, tyrosine, and histidine was identical to that reported for the Shiga toxin A subunit by Donohue-Rolfe et al. (8) on the basis of amino acid composition. The number of residues of the other amino acids predicted for SLT-1 agreed within 15% with those reported for Shiga toxin (data not shown). On the basis of the nucleotide sequence we predicted cysteine residues at positions 242 and 261, which flanked potential trypsin cleavage sites at arginine 248 and arginine 251. This is compatible with the fact that the A subunit can be cleaved into a catalytically active A1 fragment of 27 kDa and a A2 fragment of 4 kDa by reduction and treatment with trypsin (31). Residues 1 to 248 were selected arbitrarily as the A1 fragment. This sequence was compared specifically with that of several ribosomal toxins, including the α-sarcin, ricin A chain, the colicin E3 RNase domain (23), and the cloacin DF13 RNase domain (43). Other specific comparisons were with diphtheria toxin, *Pseudomonas* exotoxin A (14), cholera toxin, and *E. coli* heat-labile enterotoxin. Initial searches used the FAST P program. Significant homology was found only for the ricin A chain. The program ALIGN (7) was used to align the A1 fragment sequence with the ricin A chain (Fig. 3). Using a gap penalty of 6, the alignment score was 7.5 standard deviations above the mean score of 100 alignments of the randomly permuted sequences. The most striking similarity

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                                                    50
SLT A1     ---KEFTL-DFSTAKTYVDSLNVIRSAIGTPLQTISSGGTSLLMIDSGSG
RICIN A    IFPKQYPIINFTTAGATVQSYTNFIRAVRGRLTTGADVRHEIPVLPNRVG
                                                   100
SLT A1     DNLFAVDVRGIDPEEGRFNNLR--LIVERNNLYVTGFVNRTNNVFYRFAD
RICIN A    ---LPINQRFILVELQNHAELSVTLALSVTNAYVVGYRAGNSAYFFHPDN
                                                   150
SLT A1     ------FSHVTFPGTTAV-TLSGDSSYTTLQRVAGISRTGMQINRHSLTT
RICIN A    QEDAEAITHL-FTDVQNRYTFAFGGNYDRLEQLAGNLRENIELGNGPLEE
                                                   200
SLT A1     --SYLDLMSHSGTSLTQSVARAMLRFVTVTAEALRFRQIQRGFRTTLDDL
RICIN A    AISALYYYSTGGTQLP-TLARSFIICIQMISEAARFQYIEGEMRTRIR--
                                                   250
SLT A1     SGRSYVMTAEDVDLTLNWGRLSSVLPD-YHGQ--DSVRVGRIS--FG--S
RICIN A    YNRRSAPDPSVITLENSWGRLSTAIQESNQGAFASPIQLQRDGSKFSVYD
                         270
SLT A1     INAILGSVALILNCHHHASR--
RICIN A    VSILLPIIAMVYRCAPPPSSQF
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FIG. 3. Alignment of the predicted SLT-1 A1 fragment with the ricin A chain produced by the program ALIGN by using a gap penalty of 6. Double dots indicate identity, whereas single dots indicate conserved substitutions, as defined in the text. Dashes indicate gaps introduced in the sequences to optimize the alignment score.

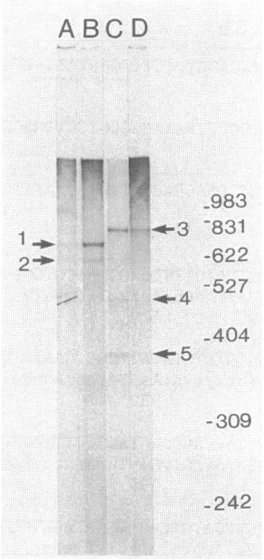

FIG. 4. S1 nuclease protection studies with total cellular RNA isolated from C600 (H-19B) (lanes A and D) and TB1(pJLB28) (lanes B and C). Probes were M13 mp18 with the sense strand of the 730-bp *Bgl*II-*Hin*dIII fragment (lanes A and B) and M13 mp18 with the sense strand of the 755-bp *Hin*cII fragment which encodes the 3′ end of the A-subunit cistron and the entire B-subunit cistron (lanes C and D). Arrows are described in the text. Numbers to the right are in base pairs.

was between residues 138 to 210 of SLT subunit A and residues 149 to 218 of ricin, in which there was 31.4% identity and conserved substitutions (as defined above) at an additional 21.4% positions in 70 possible matches. Other arbitrarily selected domains of SLT subunit A (residues 1 to 46, 95 to 137, and 211 to 248) had 18 to 22% identity and identity or conserved substitution at a total of 47 to 52% of residues aligned with the ricin A chain. Residues 47 to 94 of SLT subunit A had 22% identity but only 37% identity or conservation. When the entire SLT subunit A sequence was used, the alignment score fell to 2.95 standard deviations above the mean for 20 random alignments. The putative A2 fragment of SLT was not significantly related to the ricin A chain sequences.

RNA transcripts were examined by the S1 nuclease protection method. The 755-bp *Hin*cII fragment which encodes the C-terminal half of the A subunit and the entire B subunit was completely protected from S1 nuclease digestion by RNA isolated from both TB1(pJLB28) and the C600 (H-19B) lysogen (Fig. 4, lanes C and D, arrow 3). Additional minor protected species of 460 and 380 bp were also seen (Fig. 4, arrows 4 and 5). A total of 660 bp of the 730-bp *Bgl*II-*Hin*dIII fragment were protected from S1 nuclease digestion (Fig. 4, lanes A and B, arrow 1). Minor species, including 1 of 600 bp (Fig. 4, arrow 2), were also protected.

To identify the major promoter for the operon, primer extension experiments were performed by using total cellular RNA extracted from *E. coli* C600 (H-19B) and *E. coli* TB1(pJLB28) with a primer complementary to bases 260 to 278 of the antisense strand. A band which comigrated with chains terminated at a thymine was seen (Fig. 5). This corresponded to a message which started at position 54 on the sequence shown in Fig. 2. Computer analysis of the sequence upstream showed a −35 sequence TTGACG separated from a −10 sequence TATCAT by 17 bp (Fig. 2).

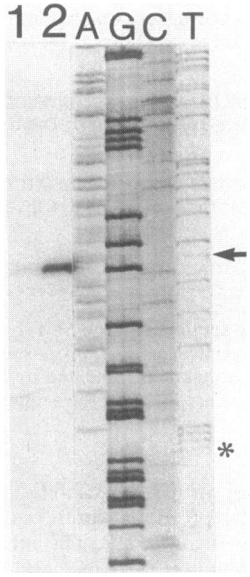

FIG. 5. Extension of a 19-mer oligonucleotide primer complementary to base pairs 260 to 278 of the antisense strand by using total cellular RNA and reverse transcriptase. RNA was extracted from C600 (H-19B) (lane 1) and TB1(pJLB28) (lane 2). Lanes A, G, C, and T are the Sanger chain-termination reactions in which the same oligonucleotide was used as a primer and the antisense strand of the *Bgl*II-*Hin*dIII fragment in M13 mp10 was used as a template. The sequence reads upward from the asterisk TTTTACGGCGCAC CGGATAAACGTAACAATAACGTAATGAAAA. A strong signal appeared in lane 2 opposite the next to last T residue (arrow), while a weaker signal was seen at the same point in lane 1.

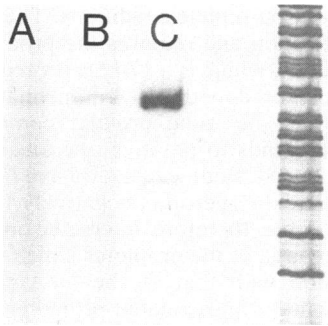

FIG. 6. Primer extensions were carried out by using RNA extracted from *E. coli* C600 (H-19B) grown in L-broth with 50 μM $FeSO_4$ (lane A), L-broth (lane B), and L-broth with 200 μM α,α'-dipyridyl (lane C). Faint primer extension species in lanes A and B comigrated with the heavy band in lane C. The A reaction lane of the Sanger dideoxy sequencing ladder in which the same primer was used is shown on the right.

These sequences agreed closely with the −35 and −10 consensus sequences of *E. coli* promoters published by Hawley and McClure (18).

The *Bgl*II-*Hin*dIII fragment pJLB28 was cloned in the vector pK01 as described above. The recombinant plasmid conferred the ability to ferment galactose and to utilize galactose as the sole carbon source on the HB101 *galK2* host.

Primer extension was also performed with RNA extracted from cultures of *E. coli* C600 (H-19B) grown under iron-starved (200 μM α,α'-dipyridyl) and iron-replete (50 μM $FeSO_4$) conditions, as well as in L-broth (6 μM Fe). Under iron-starved conditions (Fig. 6, lane C), the message started at the same site but was much more abundant than it was when cells were grown in L-broth (Fig. 6, lane B) or in iron-supplemented L-broth (Fig. 6, lane A). To show that the marked difference in quantity of the primer extension product was not an artifact, we performed other reactions in which the SLT primer and an oligonucleotide primer from the L10 operon were simultaneously extended in the same reaction mixture. No difference in the intensity of the L10 primer extension species was detected, whereas the SLT operon primer again produced a much more intense band under conditions of iron starvation (data not shown). SLT cytotoxicity was detected in the culture supernatants at dilutions of 1/256, 1/512 and 1/2,048 after growth in iron-supplemented L-broth, L-broth, and iron-starved conditions, respectively.

The program STEMLOOP was used to search for regions of dyad symmetry in sequences upstream of the A-subunit cistron. A 21-bp sequence with dyad symmetry was identified that overlapped the −10 sequence (Fig. 2). This sequence was found to match at 14 of 21 positions with a 23-bp region of dyad symmetry which overlapped the −35 region of the *iucA* promoter identified by Binderief and Neilands (2) (Fig. 7). A similar region, but one without significant dyad symmetry, was identified upstream of the *fhuA* cistron (6) by using the program BESTFIT; bases matched at 13 of 19 bases of the *iucA* and SLT dyad symmetry areas (Fig. 7). The left side of the region matched poorly. The sequence started 6 bp downstream from a putative −10 sequence. The *iucA* region matched a sequence upstream of the *fur* cistron at 14 of 19 positions (Fig. 7). By using the four sequences, a 19-bp consensus sequence was chosen which had dyad symmetry (Fig. 7).

The algorithm of Brendel and Trifonov (5) was used to identify a sequence with features of a *rho*-independent terminator. The program predicted an 8-bp stem with a 6-bp loop followed by four uracil residues. The termination site was predicted at base pair 1,673 (Fig. 2). Cloning of the *Hin*cII-*Bal*I fragment, which carries this sequence between the *gal* promoter and the *galK* gene of the vector pKG1800, reduced galactokinase production. *E. coli* C600 *galK* mutants transformed with recombinant plasmids containing this insert were unable to ferment galactose or to utilize it as a sole carbon source (data not shown).

DISCUSSION

O'Brien and LaVeck (30, 31) have shown that SLT-1 is very similar to Shiga toxin in that it has a similar mode of action and is neutralized by antiserum raised against Shiga toxin (30, 42). Newland et al. (28) have found that *S. dysenteriae* 60R contains sequences homologous to those of the cloned SLT-1 genes. In recent studies it has been shown that SLT-1 and the SLT-1 B subunit bind specifically to the glycolipid Gb_3; as was reported for Shiga-toxin (20; C. Lingwood, H. Law, S. Richardson, M. Petric, J. Brunton, and M. Karmali, J. Biol. Chem., in press). The complete identity between the amino acid sequence predicted for the SLT-1 B subunit and that determined for the Shiga toxin B subunit explains the apparent functional similarity. P pili also bind to the Gb_3 glycolipid (3). Their binding specificity differs from that of SLT, however, in that they also bind to globotetraosyl ceramide which has the *gal* α_{1-4} *gal* moiety in a subterminal position in the oligosaccharide chain (3, 20). Genetic analysis has shown that the products of the *papF* and *papG* genes are required for adhesion to the glycolipid receptor (24). There was no significant homology between the predicted amino acid sequence of the *papF* gene product and the SLT B subunit (24; data not shown).

The close agreement between the amino acid composition predicted for the A subunit of the SLT-1 genes of phage H-19B and that reported for the A subunit of Shiga toxin suggests that the two are also very closely related. Both the A1 fragment of Shiga toxin and the ricin A chain have been shown to inhibit protein synthesis by blocking peptide elongation by catalytic inactivation of the eucaryotic 60S ribosomal subunit (35). It is known that α-sarcin also inactivates the 60S ribosomal subunit and does so by cleaving the 28S rRNA of intact ribosomes at a single defined site (10). Endo et al. (9) have recently reported that ricin A chain inactivates eucaryotic ribosomes by modifying both or either of the two nucleotide residues (guanine at position 4323 and adenine at position 4324) in 28S rRNA. Their data (9) suggested that the

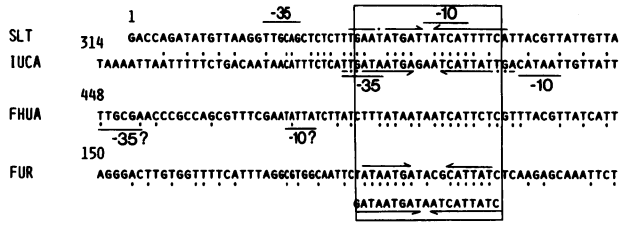

FIG. 7. Comparison of proved promoter regions of the *slt* operon and the *iucA* gene with possible promoter regions of the *fhuA* and *fur* genes. Arrows indicate areas of dyad symmetry. Numbering of the *iucA*, *fhuA*, and *fur* sequences is as described previously (2, 7, and 39, respectively). The regions of similarity of the sequences are included in the boxed area; the consensus sequence is shown at the bottom of the box. Dots indicate identity between SLT and *iucA*. The dots below the *fhuA* sequence indicate identity with SLT; those below the *fur* sequence indicate identity with the *iucA* sequence.

adenine base at position 4324 is removed, and they proposed that the toxin acts as an rRNA *N*-glycosidase. The homology that we have demonstrated between the A1 fragment of SLT-1 and ricin A chain suggests that they may have a similar mechanism of action. This appears to be the first documented instance of amino acid sequence homology between the active moiety of procaryotic and eucaryotic toxins.

The S1 nuclease protection experiments in which the 755-bp *Hin*cII fragment was used as a probe showed that the major RNA species protected a 755-bp fragment of DNA which encoded the C-terminal half of the A subunit and the entire B subunit. Thus, the A- and B-subunit cistrons are transcribed as a single unit. The additional species that were protected (470 and 380 bp) could be due to premature termination, a second weak promoter upstream from the B-subunit cistron, or both. It is impossible to distinguish between these possibilities because the probe was uniformly labeled in vivo rather than being end-labeled. In a previous study, small amounts of B subunit were produced from a plasmid construction in which the *Hin*dIII-*Bal*I fragment which carries the 3′ terminal part of the A-subunit cistron and the entire B-subunit cistron was inserted in the direction opposite to that of *lacZ* transcription in pUC18. This suggests that there is a weak promoter upstream of the B-subunit cistron (19). Insertion of the 659-bp *Hin*dIII-*Hin*cII fragment into pK01 resulted in galactokinase production, confirming that there is promoter activity in this region (J. Ginsberg, S. De Grandis, and J. Brunton, unpublished observations). It remains to be seen, however, whether the promoter functions in a normal chromosomally situated *slt* operon.

The primer extension studies showed unequivocally that the 5′ end of the SLT message is at position 54 (Fig. 2) in both the C600 (H-19B) lysogen and in the pJLB28 plasmid construction. The −35 and −10 sequences which we have proposed are very similar to the consensus sequence described by Hawley and McClure (18). It is possible, however, that the message is transcribed from a promoter which lies upstream, and the 5′ end which we identified is that of a processed species. In pJLB28 the proposed −35 sequence is only 22 bp downstream from the *Bgl*II site which was used to clone the fragment carrying the SLT operon into the pUC19 vector. In this construction the direction of SLT-1 transcription is opposite that of the *lacZ* gene (S. De Grandis and J. Brunton, unpublished data). Finally, the *Bgl*II-*Hin*dIII fragment was shown to have promoter activity by using the operon fusion vector pK01. This strongly suggests that SLT operon transcription is initiated from the promoter and start site which we have proposed. Further studies are in progress to confirm this.

Primer extension studies performed on RNA extracted from the C600 (H-19B) lysogen showed that when iron in the medium was chelated with α,α′-dipyridyl SLT transcription increased dramatically and that the same 5′ end was identified. This strongly suggests that transcription from the proposed promoter is regulated by iron. Iron chelation also resulted in increased production of SLT-1 in the extracellular medium. Production of Shiga toxin has long been known to be increased when cells are grown in medium with low iron concentrations (44), and similar observations have been made with respect to SLT (32).

Chromosomally specified iron transport systems, including the ferric-hydroxamate, ferric enterobactin, and iron dicitrate systems (11, 12, 48) and the ColV plasmid-mediated ferric aerobactin (4, 15) system, are organized as operons that are induced under iron-limited conditions and that are repressed under iron-replete conditions. The control is at the level of transcription and requires the product of the chromosomal *fur* locus, which is a 17-kDa protein (4, 16, 17, 38). Currently, the favored model for iron-mediated regulation is that iron and the *fur* gene product combine to form a repressor which binds to putative operator sites, inhibiting transcription (22, 38). Betley et al. (1) have recently reported that the H-19B toxin operon is negatively regulated by the *fur* locus. We were therefore interested in comparing the sequence in the area of the promoter which we identified by primer extension with that of the *iucA* promoter region which was the first iron-regulated promoter to be identified by direct methods (2). The homologous areas of dyad symmetry found in the *iucA* and *slt* operon promoter regions would appear to be excellent candidates for repressor-binding sites. The proposed operator site upstream of the *fhuA* gene (which codes for the ferrichrome receptor) does not have dyad symmetry, and there is significant divergence from the other regions in the left-hand 7 bp (Fig. 6). This may account for the finding that *fhuA* repression requires iron concentrations higher than those that repress the *fepA* (enterobactin receptor) locus (16). The presence of another similar sequence upstream of the *fur* cistron suggests that this gene may be autoregulated.

A potential *rho*-independent terminator structure was identified 263 bp downstream from the terminal arginine codon of the B subunit. It seems likely that this functions as the terminator in vivo, but we have no direct evidence for this. The 600-bp *Hin*cII-*Bal*I fragment that carries this sequence was shown to have terminator activity in the pKG1800 vector system. The terminator activity was not, however, precisely localized on this fragment.

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ADDENDUM IN PROOF

The sequence of the SLT operon of bacteriophage 933J has recently been published (M. Jackson, J. Newland, R. Holmes, and A. O'Brien, Microb. Pathogenesis **2**:147–153, 1987), as has that of H-19B (S. Calderwood, F. Auclair, A. Donohue-Rolfe, G. Keusch, and J. Mekalanos, Proc. Natl. Acad. Sci. USA **84**:4364–4368).

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