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Cloning and Expression of the Genes Specifying Shiga-Like Toxin Production in *Escherichia coli* H19

ANNIE HUANG,[2] STEPHANIE DE GRANDIS,[2] JAMES FRIESEN,[3] MOHAMMED KARMALI,[2,5] MARTIN PETRIC,[2,5] ROSA CONGI,[2] AND JAMES L. BRUNTON[1,2,4]*

Departments of Medicine,[1] Microbiology,[2] and Medical Genetics,[3] University of Toronto; Mount Sinai Hospital[4]; and The Hospital for Sick Children,[5] Toronto, Ontario, M5G 1X5 Canada

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Some strains of *Escherichia coli* produce a protein which is cytotoxic for Vero cell and HeLa cell monolayers. This toxin is very similar to the toxin of *Shigella dysenteriae* 1 and has been named verotoxin or *E. coli* Shiga-like toxin. It has been shown that toxin conversion is due to a group of bacteriophages, one of which has been designated H-19B. In this study we report hybridization experiments showing that part of the H-19B genome is homologous to phage lambda. We have cloned a 1.7-kilobase *BalI-BglII* fragment from the genome of H-19B into pUC18. The recombinant plasmid confers the ability to produce high levels of Shiga-like toxin on transformed *E. coli* cells. We demonstrate using an in vitro transcription/translation system that the cloned fragment specifies the two verotoxin subunit peptides which have masses of 31 and 5.5 kilodaltons. The identity of peptides was confirmed by immunoprecipitation with verotoxin antiserum and protein A-Sepharose beads.

Konowalchuk et al. (10) originally reported that certain strains of *Escherichia coli* produce a heat-labile protein, designated verotoxin (VT), which has a cytotoxic effect in Vero cell monolayers. O'Brien et al. (17) showed that this toxin is immunologically very similar to the Shiga toxin of *Shigella dysenteriae* type 1. The subunit structure, isoelectric point, and biological activities were also similar (18), and it was suggested that VT should be renamed *E. coli* Shiga-like toxin (SLT) (A. D. O'Brien, T. Lively, M. Chen, S. Rothman, and S. Formal, Letter, Lancet i:702, 1983). Recently, Karmali et al. (7) showed that VT-producing *E. coli* (VTEC) correspond to the high-level SLT-producing *E. coli* of O'Brien et al. (19). Canadian and British workers continue to use the name "verotoxin," whereas American workers use the term "*E. coli* Shiga-like toxin."

A number of recent studies have suggested that VTEC are an important cause of human disease. Associated diarrheal illnesses have included undifferentiated diarrhea (21) and hemorrhagic colitis (W. M. Johnson, H. Lior, and G. Bezanson, Letter, Lancet i:76, 1983; O'Brien et al., Letter); the latter is associated with *E. coli* serotype O157:H7. Karmali et al. (8) recently demonstrated a close association between VTEC infection and the hemolytic uremic syndrome and suggested that VT is a precipitating cause of the microvascular thrombosis which is characteristic of that disease.

Smith et al. (23) have recently shown that some VTEC strains carry a temperate bacteriophage which can confer the toxigenic property on *E. coli* C600. They described two bacteriophages, H-19A and H-19B, which were isolated from serogroup O26 *E. coli* H19 and could independently confer toxigenicity on *E. coli* C600. O'Brien et al. (20) also described two bacteriophages, 933J and 933W, which were isolated from an *E. coli* O157:H7 strain. Moreover, they found that phage-mediated toxin conversion resulted in the production of high levels of toxin. Determining the true role of SLT in human disease has been complicated by the finding that many *E. coli* strains produce minute to moderate amounts of SLT under certain conditions (19).

* Corresponding author.

In view of the reported ubiquity of SLT production in organisms which cause diarrhea (19), we sought to determine whether the bacteriophages simply exerted positive regulation on structural genes present in all *E. coli* strains or whether the phages carried the structural genes of the toxin. Because detection of SLT is time-consuming and tedious, even when simple assays are used, we also thought that a diagnostic DNA hybridization probe might be helpful in defining the epidemiology of these organisms. As an initial approach to these problems, we report here the cloning of a 1.7-kilobase-pair (kb) *BalI-BglII* fragment from phage H-19B which specifies SLT production.

(This study was presented in part at the 84th Annual Meeting of the American Society for Microbiology, Las Vegas, Nevada, in March 1985 and at the 25th Interscience Conference on Antimicrobial Agents and Chemotherapy, Minneapolis, Minnesota, in October 1985.)

MATERIALS AND METHODS

Strains and media. Strains of *E. coli* C600 carrying the toxin-converting phages H-19B, H-19A, H-12, and H-23 were obtained from H. Williams Smith (Houghton Poultry Research Station, Huntingdon, Cambridgeshire, United Kingdom). *E. coli* TB1 *lac pro rpsL ara thi* φ80 d *lacZΔM15 hsdR* was obtained from Bethesda Research Laboratories and was used as the host for the pUC vectors and recombinant plasmids. The identity of phage H-19B was confirmed by demonstrating that it formed plaques on *E. coli* strains C600, C600 (H-12), and C600 (H-23) but not on *E. coli* C600 (H-19A) or *E. coli* C600 (H-19B) (23). Plasmids pUC9, pUC18, and pUC19 were obtained from J. Messing (13). Strains were grown in L broth (15) or brain heart infusion, supplemented as necessary with carbenicillin (50 μg/ml) and 5-bromo-4-chloro-3-indolyl-β-galactopyranoside (50 μg/ml; Boehringer Mannheim Biochemicals).

DNA preparation. Bacteriophage H-19B DNA was prepared from purified phage stocks by a method used for purification of phage lambda DNA (12). Low-titer phage stocks were prepared from overnight cultures of the lysogen *E. coli* C600 (H-19B) in brain heart infusion supplemented with 3 mM calcium chloride and 3 mM magnesium chloride.

Cell lysis was completed by the addition of chloroform to the overnight culture. The cultures were then centrifuged, and the supernatant was sterilized by filtration with a 0.45-μm (pore size) Millipore filter. High-titer (10^{12}/ml) stocks of phage were prepared by infecting *E. coli* C600 with phage H-19B at a multiplicity of infection of 0.01 followed by incubation in a soft agar overlay until confluent lysis occurred. Phage were harvested by diffusion into lambda phage buffer (12), concentrated by precipitation with polyethylene glycol 6000, and purified by isopycnic centrifugation in cesium chloride gradients as described by Yamamoto et al. (26).

Plasmid DNA was prepared using the method of Birnboim and Doly (1). Further purification was achieved by ultracentrifugation in cesium chloride-ethidium bromide gradients (12). Plasmid DNA was electrophoresed in 0.7 to 1.5% agarose gels using a Tris-borate-EDTA buffer (14).

Restriction mapping and cloning. Restriction endonucleases were purchased from Boehringer Mannheim, and digestions were performed according to the manufacturer's instructions. Phage lambda DNA digested with *Hin*dIII or *Eco*RI-*Hin*dIII was used as the molecular weight standard (22). Restriction mapping was performed by a variety of methods including double digestions, partial digestion, and digestion of isolated fragments. Sometimes conclusions were confirmed by hybridization. In most cases, restriction fragments were extracted from low-melting-temperature agarose gels (Bio-Rad Laboratories) as described by Maniatis et al. (12). Ligations were carried out overnight at 14°C using 1 U of T4 ligase per 20 μl of reaction mixture.

Southern blotting and hybridization. Southern blotting and hybridization was performed in the standard manner (24). Hybridizations were performed at 37°C in 50% formamide–2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–10× Denhardt solution (5). After hybridization, the filters were washed with 1 × SSC at 68°C for 1 h.

Plasmid-specified peptides were produced by using a procaryotic in vitro DNA transcription/translation system purchased from Amersham Canada, Ltd. (no. 3802; Oakville, Ontario). Approximately 1 to 1.5 μg of purified plasmid DNA was used for each reaction, and 9 μCi of [^{35}S]methionine was added per sample. pUC18 DNA carrying no insert was used as a control. Reactions were performed according to the manufacturer's instructions and were stopped by cooling on ice. One volume of 2% sodium dodecyl sulfate–4% 2-mercaptoethanol–0.625 M Tris (pH 6.8)–10% glycerol was added, and the mixture was boiled for 5 min before loading onto a 15% sodium dodecyl sulfate-polyacrylamide gel with a 5% stacking gel (11).

Immunoprecipitation. The immunoprecipitation was performed as described by Kessler (9) by using a 1/10 dilution of SLT antiserum adsorbed against *E. coli* TB1(pUC18) and protein A-Sepharose CL4B beads (Sigma Chemical Co.). Beads were washed at 4°C with buffer composed of 10 mM Tris (pH 7.5), 0.1% Triton X-100, 2 mM EDTA, 100 μM phenylmethylsulfonyl fluoride, 0.15 or 0.5 M NaCl, and bovine serum albumin (1 mg/ml). Competition experiments were performed by pretreating the antiserum with 1.5×10^6 50% tissue culture cytotoxic dose units of purified unlabeled SLT at 4°C for 30 min before immunoprecipitation. One such unit is the amount of toxin which produces a cytopathic effect on 50% of the cells of a monolayer after 72 h of incubation. After electrophoresis, gels were fixed and saturated with En^{3}Hance (New England Nuclear Corp.) before being dried on paper and subjected to fluorography using Kodak XAR5 film.

Bioassay of SLT. *E. coli* strains carrying recombinant plasmids were assayed qualitatively for SLT production as previously described (8). Selected strains were assayed in a semiquantitative manner by testing serial twofold dilutions (in phosphate-buffered saline) of the supernatant of overnight Penassay broth (Difco Laboratories) cultures for cytotoxicity in the Vero cell system as described by Karmali et al. (8). SLT was purified to homogeneity from *E. coli* strain H-30 as described elsewhere (M. Petric, M. Karmali, R. Cheung, and S. Louie, manuscript in preparation). Rabbits were immunized with glutaraldehyde-inactivated toxin by the method of Brown et al. (2).

RESULTS

*Eco*RI cleavage of phage H-19B DNA yielded 12 fragments. The quantity of two of the fragments, one 1.2 kb and the other 5 kb, was increased by heating the digests to 65°C, followed by cooling on ice before loading the gel. The resultant increase in DNA of the 5- and 1.2-kb fragments was accompanied by a reduction in the intensity of a 6.2-kb band (A. Huang, J. Friesen, and J. Brunton, manuscript in preparation). We interpreted this as evidence that the 5- and 1.2-kb fragments carry cohesive termini and used them as markers to construct a restriction endonuclease map of the phage genome (Fig. 1A). *Eco*RI fragments of H-19B DNA were isolated as described and were individually ligated to pUC9 and transformed into *E. coli* TB1. A strain carrying the 8.1-kb *Eco*RI fragment (pJLB2; Fig. 1B) produced SLT which was neutralized by specific antiserum (data not shown). Experiments were carried out to determine the extent of homology between the lambda and H-19B phages. Nick-translated total phage lambda DNA was used in hybridization experiments. The 2.7-kb *Hin*dIII-*Eco*RI fragment cut from the insert of pJLB2 is strongly homologous to phage

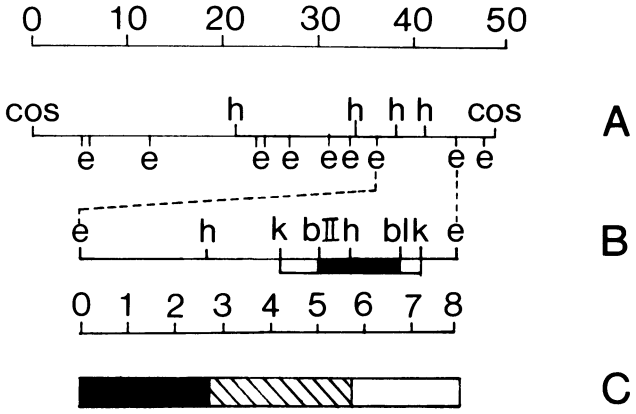

FIG. 1. (A) Restriction map of phage H-19B obtained by using *Eco*RI and *Hin*dIII. The termini are the cohesive termini (cos). The sizes (in kb pairs) of the *Eco*RI fragments from the left are 5.0, 1.0, 6.2, 11.0, 0.9, 2.5, 4.0, 2.0, 2.7, 8.1, 3.0, and 1.2. The sizes of the *Hin*dIII fragments from the left are 20, 12.5, 4.0, 3.0, and 7.0. Two *Hin*dIII fragments of 0.7 and 0.3 kb have been omitted from the map. (B) Map of 8.1-kb toxin-positive insert of pJLB2 showing *Hin*dIII, *Bgl*II, *Kpn*I, and *Bal*I sites. The open bar indicates the 3-kb *Kpn*I fragment which was sublcloned. The solid bar indicates the 1.7-kb *Bal*I-*Bgl*II fragment which specified SLT production. (C) *Eco*RI-*Hin*dIII-generated fragments of pJLB2 insert showing strong (■), weaker (▨), and no (□) hybridization when nick-translated phage lambda DNA was used as a probe (see Fig. 2 for additional data). Map distances are given in kb pairs. Abbreviations: e, *Eco*RI; bII, *Bgl*II; k, *Kpn*I; h, *Hin*dIII; bl, *Bal*I; cos, cohesive termini.

lambda (Fig. 2, lanes D and d), whereas there was less hybridization to the 3.0-kb *Hin*dIII fragment. There was no hybridization to the 2.3-kb *Eco*RI-*Hin*dIII fragment. Reciprocal hybridizations using nick-translated pJLB2 DNA showed that the cloned fragment of H-19B was homologous only to the 6.6-kb *Hin*dIII fragment of phage lambda (data not shown). Figure 1C shows the region of the *Eco*RI insert which hybridized with phage lambda in relation to the restriction map of the pJLB2 insert and phage H-19B.

On the basis of the results shown in Fig. 2, a 3-kb *Kpn*I fragment (as indicated in Fig. 1B) was subcloned into pUC18; the recombinant was designated pJLB5. Transformants produced active toxin which could be neutralized with specific antiserum (data not shown). Fragments of this *Kpn*I insert were subcloned and tested for SLT production in the tissue culture assay. The results are summarized in Fig. 3. Only plasmids pJLB11 and pJLB28, which carried the *Kpn*I-*Bgl*II and *Bal*I-*Bgl*II inserts, respectively, produced biologically active toxin. In contrast, the pJLB15 (*Kpn*I-*Hin*dIII) and pJLB17 (*Hin*dIII-*Bgl*II) inserts did not specify production of biologically active toxin.

To determine which peptides were specified by the various inserts, plasmid DNA was purified and used to direct in vitro protein synthesis. Figure 4 shows a fluorograph of a polyacrylamide gel electrophoresis analysis of [^{35}S]methionine-labeled peptides produced by various subclones. The *Kpn*I fragment in pJLB5 produced two peptides with molecular masses of 31K (31,000 molecular weight) and 5.5K (lane C).

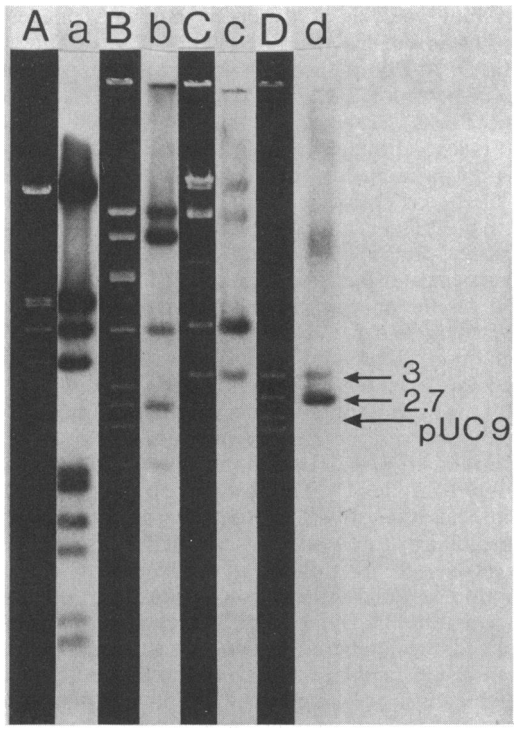

FIG. 2. pJLB2, phage H-19B, and phage lambda DNAs were digested with restriction endonucleases, electrophoresed on a 0.7% agarose gel, and transferred to nitrocellulose. Nick-translated total phage lambda DNA was used as a probe for hybridization. Lanes: A and a, phage lambda digested with *Eco*RI and *Hin*dIII; B and b, phage H-19B digested with *Eco*RI; C and c, phage H-19B digested with *Hin*dIII; D and d, pJLB2 digested with *Hin*dIII and *Eco*RI; A to D, stained gels; a to d, autoradiograms.

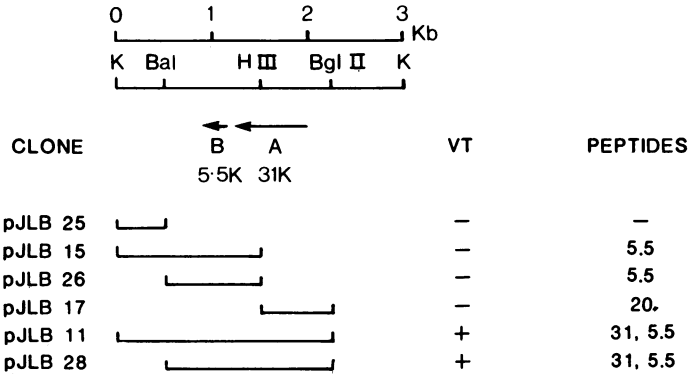

FIG. 3. Restriction enzyme map of 3.0-kb VT-positive *Kpn*I fragment cloned in pJLB5 and fragments which were subcloned from it. The SLT phenotype (indicated as VT and determined by the cytotoxicity assay) and the peptides produced (determined by polyacrylamide gel electrophoresis of the products of in vitro protein synthesis directed by pUC18 carrying the relevant insert) are also indicated. Arrows indicate the proposed location and direction of transcription of the cistrons for the A and B subunits. Peptide sizes are indicated in kilodaltons.

The same peptides were produced by the *Kpn*I-*Bgl*II (pJLB11) and *Bal*I-*Bgl*II (pJB28) fragments (data not shown). Clones carrying the *Kpn*I-*Hin*dIII (pJLB15; Fig. 4, lane D) and *Bal*I-*Hin*dIII (pJLB26; lane E) fragments produce only the 5.5K peptide in an amount which appears to be less than that produced by the *Kpn*I fragment. In contrast, the *Hin*dIII-*Bgl*II subclone (pJLB17; lane F) produces a large amount of a 20K peptide. This 20K peptide is not produced by the 3-kb *Kpn*I fragment or by any of the other clones which yield biologically active SLT. These results are summarized in Fig. 3 in relation to the map of the 3.0-kb *Kpn*I fragment.

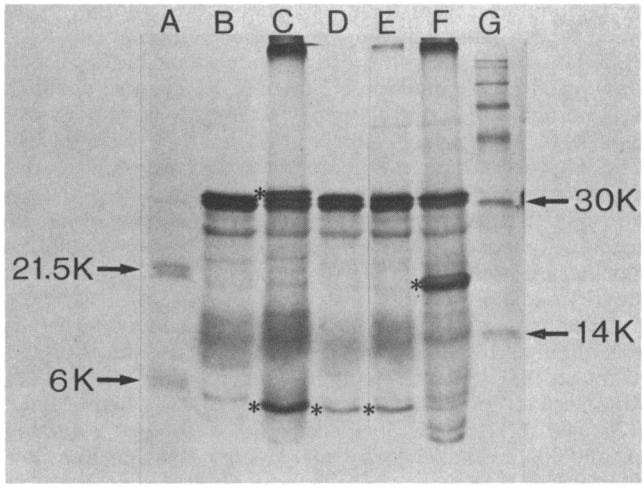

FIG. 4. Fluorogram of [^{35}S]methionine-labeled peptides produced by the in vitro protein synthesis system directed by plasmid DNA. Lane A: molecular-weight standards carbonic anhydrase (30K), trypsin inhibitor (21K), cytochrome *c* (12.5K) and aprotinin (6.5K). Lanes B to F: in vitro products pUC18 (lane B), pJLB5 (lane C), pJLB15 (lane D), pJLB26 (lane E), and pJLB17 (lane F). Lane G: molecular-weight standards myosin (200K), phosphorylase *b* (92K), bovine serum albumin (69K), ovalbumin (46K), and lysozyme (14K). The novel peptides are marked with an asterisk: lane C, 5.5K and 31K; lane D, 5.5K; lane E, 5.5K; and lane F, 20K.

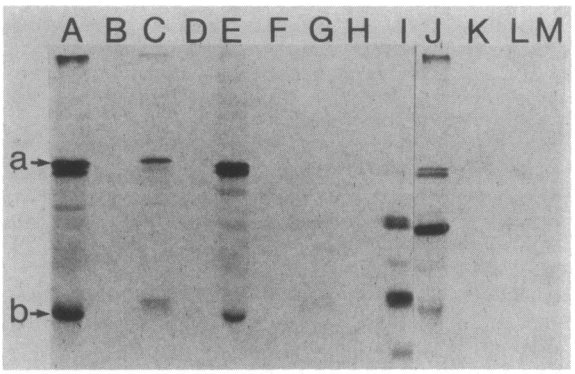

FIG. 5. Immunoprecipitation of [^{35}S]methionine-labeled peptides produced by in vitro protein synthesis directed by plasmid DNA. Peptides were immunoprecipitated using protein A-Sepharose CL4B beads and SLT antiserum. Specificity was confirmed by controls using preimmune serum and by competition with purified unlabeled SLT. Lanes A to D (pJLB11): total protein (A) precipitated with preimmune serum (B) or SLT antiserum (C) or in competition with unlabeled SLT (D). Lanes E to H (pJLB15): total protein (E) precipitated with preimmune serum (F) or SLT antiserum (G) or in competition with unlabeled SLT (H). Lanes J to M (pJLB17): total protein (J) precipitated with preimmune serum (K) or SLT antiserum (L) or in competition with unlabeled SLT (M). a, mobility of 31K A subunit; b, mobility of 5.5K B subunit.

The identity of the peptides was confirmed by immunoprecipitation. Antiserum initially raised in rabbits against purified SLT was adsorbed with *E. coli* TB1(pUC18) and was used for the immunoprecipitation of in vitro-synthesized peptides. The 31K and 5.5K peptides were immunoprecipitated by SLT antiserum but not by preimmune serum (Fig. 5, lanes B and C). Immunoprecipitation of labeled peptides was blocked by preincubation with an excess of unlabeled purified SLT (lane D). Similarly, the 5.5K peptide specified by pJLB15 was precipitated by SLT antiserum (lane G) but not by preimmune serum (lane F); immunoprecipitation of labeled peptide was again blocked by the addition of unlabeled SLT (lane H). Finally, the 20K peptide specified by pJLB17 was specifically immunoprecipitated by SLT antiserum (lane L), and again the controls (lanes K and M) were negative. The 5.5K band which appeared as a sharp band before immunoprecipitation ran more diffusely and slowly after immunoprecipitation. We are unable to explain this finding. The fact that the 5.5K, 31K, and 20K peptides could be specifically precipitated and that precipitation could be blocked by preincubation with purified SLT confirms their identity as SLT subunits or derivatives thereof.

An estimate of the amount of toxin produced by the clones was obtained by testing serial dilutions of the sterile supernatant in the Vero cell assay system. *E. coli* TB1(pJLB11) supernatants produced a cytotoxic effect when diluted 1/260,000. TB1(pJLB28) supernatant was toxic at a dilution of 1/30,000, while *E. coli* C600(H-19B) was positive at a dilution of 1/1,024.

DISCUSSION

The preliminary hybridization studies presented here show that part of the phage H-19B genome is homologous to the 6.6-kb *Hin*dIII fragment of the lambda genome. The latter fragment includes the *c*I and *ninR* genes, as well as the origin of vegetative replication and the *O* and *P* genes required for DNA replication (6). The area of homology is currently being mapped in more detail, and studies of other homologous areas of the phages are in progress (Huang et al., in preparation). O'Brien et al. (19) found that, in contrast to the large amounts of extracellular SLT produced by O157:H7 strains and strain H19, *E. coli* K-12 (and many other *E. coli* strains) produced very small to moderate amounts of SLT which could only be detected by the testing supernatants derived from the disruption of several grams (wet weight) of cells grown in iron-free medium. It therefore seemed possible that the converting phage might exert a regulatory effect on the SLT genes either by producing a diffusible regulator or by a *cis* effect which was dependent on integration at a particular site in the *E. coli* chromosome. In this study we demonstrate that the production of high levels of SLT is determined by a 1.7-kb *Bal*I-*Bgl*II fragment of the phage H-19B genome. When this fragment was used to direct protein synthesis in vitro, 31K and 5.5K peptides were produced and immunoprecipitated by anti-SLT antiserum. This proves that the toxin conversion is due to the presence of the structural genes for the SLT subunits on the phage H-19B genome. Newland et al. (16) recently reached a similar conclusion following the cloning of the SLT structural gene from phage 933J, which was isolated from an *E. coli* O157:H7 strain. Interestingly, the size and ordering of all but the two smallest *Hin*dIII fragments of phage 933J (16) are similar to the map of phage H-19B (Fig. 1). The location of the toxin genes also appears to be similar, indicating that the two genomes are very closely related. Willshaw et al. (25) recently reported cloning the SLT genes of phage H-19 and mapped their location by insertional inactivation. Neill et al. have also presented indirect evidence for the presence of SLT structural genes on the converting phage by using Western blot analysis of lysogens carrying phages with various Tn*5* insertions (R. J. Neill, N. R. Serrano, S. W. Rothman, D. A. Foret, and D. H. Wells, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, B112, p. 36).

The *Hin*dIII-*Bgl*II insert of pJLB17 produced a 20K peptide that is also immunoprecipitated by SLT antiserum, but which is not produced by the larger *Bal*I-*Bgl*II fragment or by any of the other recombinants which produced biologically active SLT (Fig. 3). We conclude from this that about two-thirds of the 31K A subunit is coded on the *Hin*dIII-*Bgl*II fragment and that transcription is from right to left as indicated on the map in Fig. 3. This results in the 20K peptide, which is a truncated version of the 31K protein. Although it is difficult to control the amount of protein produced by plasmids in the in vitro protein synthesis system, it did seem that reproducibly smaller amounts of the 5.5K peptide were produced from clones carrying the *Bal*I-*Hin*dIII and *Kpn*I-*Hin*dIII segments than from clones carrying the *Kpn*I fragment. This observation is consistent with the hypothesis that the B subunit is transcribed from the same promoter as the A subunit, but that it also has its own weaker promoter. If this is true, the order of subunit transcription would be the same as for *E. coli* heat-labile enterotoxin although Dallas et al. (4) reported that recombinant plasmids which did not produce A subunit produced no B subunit at all. Newland et al. (16) also found that a 20K protein which was presumed to be a truncated version of the 31K A subunit was produced from sequences on one side of the internal *Hin*dIII site, while the B subunit peptide was specified by sequences on the other side. The cloning and characterization of the H-19B SLT genes opens the way to determining whether low-level SLT-producing organisms carry genes homologous to the VT genes carried on phage H-19B and differ only with respect to the regulation of their expression.

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LITERATURE CITED

1. **Birnboim, H., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513–1523.
2. **Brown, J. E., D. E. Griffin, S. W. Rothman, and B. P. Doctor.** 1982. Purification and biological characterization of Shiga toxin from *Shigella dysenteriae* 1. Infect. Immun. **36**:996–1005.
3. **Cohen, S. N., A. C. Chang, and H. Hsu.** 1972. Nonchromosomal antibiotic resistance in bacteria: genetic transformation of *Escherichia coli* by R factor DNA. Proc. Natl. Acad. Sci. USA **69**:2110–2114.
4. **Dallas, W. S., D. M. Gill, and S. Falkow.** 1979. Cistrons encoding *Escherichia coli* heat-labile toxin. J. Bacteriol. **139**:850–858.
5. **Denhardt, D. T.** 1966. A membrane filter technique for the detection of complementary DNA. Biochem. Biophys. Res. Commun. **23**:641–646.
6. **Hendrix, R., J. Roberts, F. Stahl, and R. Weisberg (ed.).** 1983. Lambda II. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
7. **Karmali, M. A., M. Petric, C. Lim, R. Cheung, and G. Arbus.** 1985. Sensitive method for detecting low numbers of verotoxin-producing *Escherichia coli* in mixed cultures by use of colony sweeps and polymyxin extraction of verotoxin. J. Clin. Microbiol. **22**:614–619.
8. **Karmali, M. A., M. Petric, C. Lim, P. Fleming, G. Arbus, and H. Lior.** 1985. The association between idiopathic hemolytic uremic syndrome and infection by verotoxin-producing *Escherichia coli*. J. Infect. Dis. **151**:775–782.
9. **Kessler, S. W.** 1981. Use of protein A-bearing staphylococci for the immunoprecipitation and isolation of antigens from cell. Methods Enzymol. **73**:452–453.
10. **Konowalchuk, J., J. Speirs, and S. Stavric.** 1977. Vero cell response to a cytotoxin of *Escherichia coli*. Infect. Immun. **18**:775–779.
11. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
12. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
13. **Messing, J., and J. Vieira.** 1982. A new pair of M13 vectors for selecting either DNA strand of double-digest restriction fragments. Gene **19**:269–276.
14. **Meyers, J. A., D. Sanchez, L. Elwell, and S. Falkow.** 1976. Simple agarose gel electrophoresis method for identification and characterization of plasmid deoxyribonucleic acid. J. Bacteriol. **127**:1529–1537.
15. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
16. **Newland, J., N. Strockbine, S. Miller, A. O'Brien, and R. K. Holmes.** 1985. Cloning of the Shiga-like toxin structural genes of a toxin converting phage of *Escherichia coli*. Science **230**:179–181.
17. **O'Brien, A. D., and G. D. LaVeck.** 1982. Immunochemical and cytotoxic activities of *Shigella dysenteriae* 1 (Shiga) and Shiga-like toxins. Infect. Immun. **35**:1151–1154.
18. **O'Brien, A. D., and G. D. LaVeck.** 1983. Purification and characterization of a *Shigella dysenteriae* 1-like toxin produced by *Escherichia coli*. Infect. Immun. **40**:675–683.
19. **O'Brien, A. D., G. D. LaVeck, M. Thompson, and S. Formal.** 1982. *Shigella dysenteriae* 1 (Shiga)-like cytotoxin produced by *Escherichia coli*. J. Infect. Dis. **146**:763–769.
20. **O'Brien, A. D., J. Newland, S. Miller, R. Holmes, H. Williams Smith, and S. Formal.** 1984. Shiga-like toxin-converting phages from *Escherichia coli* strains that cause hemorrhagic colitis or infantile diarrhea. Science **226**:694–696.
21. **Rowe, B.** 1981. Enteropathogenic *Escherichia coli* (EPEC): Importance and pathophysiology, p. 101–106. *In* T. Holme, J. Holmgren, M. H. Menson, and R. Mollby (ed.), Acute enteric infections in children. New Prospects for treatment and prevention. Elsevier/North-Holland Biomedical Press, Amsterdam.
22. **Sanger, F., A. Coulson, G. Hong, D. Hill, and G. Petersen.** 1982. Nucleotide sequence of bacteriophage lambda DNA. J. Mol. Biol. **162**:729–773.
23. **Smith, H. W., P. Green, and Z. Parsell.** 1983. Vero cell toxins in *Escherichia coli* and related bacteria: transfer by phage and conjugation and toxic action in laboratory animals, chickens, and pigs. J. Gen. Microbiol. **129**:3121–3137.
24. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
25. **Willshaw, G. A., H. R. Smith, S. Scotland, and B. Rowe.** 1985. Cloning of genes determining the production of Vero cytotoxin by *Escherichia coli*. J. Gen. Microbiol. **131**:3047–3053.
26. **Yamamoto, K. R., B. Alberts, R. Benzinger, L. Lawhorne, and G. Treiber.** 1970. Rapid bacteriophage sedimentation in the presence of polyethylene glycol and its application to large-scale virus purification. Virology **40**:734–744.