JOURNAL OF BACTERIOLOGY, Dec. 1978, p. 1165-1173
0021-9193/78/0136-1165$02.00/0

Vol. 136, No. 3
Printed in U.S.A.

*Hin*dII and *Hin*dIII Restriction Maps of the *attφ80-tonB-trp* Region of the *Escherichia coli* Genome, and Location of the *tonB* Gene

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Received for publication 15 June 1978

The *Hin*dII and *Hin*dIII restriction maps of the *attφ80-tonB-trp* region of the *Escherichia coli* chromosome are presented. Analysis of phage DNAs carrying *tonB* mutations has allowed identification of a 1,730-base pair *Hin*dII fragment containing at least part of the *tonB* gene. This fragment is 4,020 base pairs from the end of *trpA*, with the total distance from *attφ80* to *trpA* being 6,550 ± 800 base pairs. Properties of hybrid plasmids containing insertions of various $tonB^+$ restriction fragments suggest that *tonB* lies completely within the 1,730-base pair fragment. In addition, apparent fusions of β-galactoside to proteins within the *tonB* region suggest that the entire region codes for more than one polypeptide.

The region of the *Escherichia coli* genetic map between *attφ80* and *trpA* at about 27 min (1) is known to code for only one gene: *tonB*. Mutants in *tonB* are resistant to bacteriophages T1 and φ80, tolerant to many colicins, defective in high-affinity iron transport (19), and defective in vitamin B_{12} transport (5). Since the region between *attφ80* and *trpA* is estimated to be ≥6,500 base pairs (bp) long (12), one might anticipate finding more than one gene in the *tonB* region. A maximum estimate of protein coding capacity, ignoring the possible existence of regulatory elements, is nearly 270,000 daltons.

The lack of other mutations in the *tonB* region necessitates a nongenetic approach to characterizing the region. One strategy for this is comparison of a restriction map of the region with analysis of proteins coded by defined fragments of DNA within the region. In this report we describe a *Hin*dII and *Hin*dIII restriction analysis of transducing phages carrying various parts of the *attφ80-tonB-trp* region. *tonB* deletions have enabled us to pinpoint one *Hin*dII fragment which carries at least part of the *tonB* gene. Preliminary attempts to define the $tonB^+$ region coding for the phenotype through cloning into ColE1 plasmids are also presented. In addition, we have isolated several fusions of β-galactosidase to proteins within the *tonB* region, indicating that the region codes for more than one polypeptide.

MATERIALS AND METHODS

Buffers and media. *Hin*d buffer, polyacrylamide gel electrophoresis buffer, phage buffer, λCa buffer, M63 buffer, T-agar, F-top agar, and Trp agar have all been described elsewhere (4, 11, 12). Agarose gel electrophoresis buffer is 0.16 M tris(hydroxymethyl) aminomethane (Tris)-acetate (pH 8.3)-0.08 M sodium acetate-8 mM disodium ethylenediaminetetraacetate (EDTA)(3). Amplification medium for growth of plasmids consists of M9 buffer containing 0.2% Casamino Acids, 0.4% glucose, 4 μg of vitamin B_1 per ml, and 0.001 M $MgSO_4$. Ligation buffer is 0.02 M Tris-hydrochloride (pH 7.6)-0.01 M $MgCl_2$-0.001 M dithiothreitol-0.001 M EDTA-50 μM ribo-ATP (23). SSC-$CaCl_2$ used in transfections is 1 volume of SSC (0.15 M NaCl, 0.015 M trisodium citrate, pH 7.0) plus 2 volumes of 0.1 M $CaCl_2$. Chromium agar is 15 g of agar per liter in M9 supplemented with 0.3% Casamino Acids, 0.2% glucose, 4 μg of B_1 per ml, 0.01 M $MgSO_4$, and 100 μM $CrCl_3$. Neomycin was used at a concentration of 50 μg/ml in agar plates.

Bacteria and bacteriophages. All strains of bacteria and bacteriophages used are listed in Table 1. Preparation of φ80*vir* and colicin V+B has been described elsewhere (9).

Phage f2#5 and other $tonB^-$ f2-derived phages were constructed as follows. Bacterial strain Ymel was lysogenized with λ*attφ80*c*lts2N7N53Δnin*1 (hereafter called phage f2). At 30°C, independent cultures of Ymel·f2 were treated with colicin V+B and φ80*vir* to select *tonB* mutants as described by Gottesman and Beckwith (9). These *tonB* lysogens were induced at 42°C, and the resultant lysates were screened for $trpA^+$ phage with Ymel ΔA on Trp media. Purified plaques were screened for the *N*-dependent expression of the *trpA* gene, indicating that they did not carry the entire *trp* operon (11). Phage f2*trpA* was constructed by inducing Ymel lysogenic for f2, without previous *tonB* selection, and screening among $trpA^+$ plaques for one which did not carry $trpB^+$ by a complementation test.

λ*ptrp-lac*K6 and other phages carrying $tonB^-$ lac^+ fusions were constructed as follows. An F′ *lacZ118* (formerly $lacZ^-_{U118}$; 16) was transferred into strain

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TABLE 1. *Bacteria and bacteriophage strains used in this study*

Strain	Pertinent characteristics	Source or reference
Bacterial		
Ymel	F⁻ *supF*	L. Hallick
Ymel ΔA	F⁻ Δ(*tonB-trpA*)*553 supF*	Δ(*tonB-trpA*)*553* (obtained from C. Yanofsky) was introduced into Ymel by P1*vir* transduction
X7800 (*lacZ118*)	F⁻ φ80d*lac* (*lacZ118*) Δ(*lac-pro*) Su⁻ *trpR*	X7800 (14) homogenotized with F′ *lacZ118*
pRZ112/MO	F⁻ Sm^r harboring plasmid pRZ112	R. Jorgensen
W205	F⁻ φ80d*lac* Δ(*trp-tonB-lac*)W205 Δ(*lac-pro*) Su⁻	W. Reznikoff (14)
Bacteriophage		
f2	λ*attφ80cIts2N7N53Δnin*1	N. Franklin via K. Carlsen
13 4/6 5	f2p*tonB*⁺ *trpABDCEop*⁺	B. Jones (12)
λp*trp-lac*W2*nin*1	λ Transducing phage carrying W2 *trp-lac* fusion	λp*trp-lac*W2 (4) *nin*1 deletion crossed on subsequently

X7800. This early *lacZ* nonsense mutation was crossed onto the chromosome by homogenotization. *tonB* derivatives of X7800 (*lacZ118*) were selected by using 25 ml of a saturated culture each time as described above and were screened for *lacZ*⁺ colonies on nutrient plates containing the indicator dye XG (5 bromo-5-chloro-3-indolyl-β-D-galactoside).

tonB lacZ⁺ colonies were presumed to have arisen by simultaneous deletion of the *118* mutation in *lacZ* and deletion of all or part of *tonB*. Double events such as reversion of *118* or mutation to an ochre nonsense suppressor along with simultaneous *tonB* mutation would be exceedingly rare. The effect of these deletions is to fuse β-galactosidase, the *lacZ* gene product, to other proteins in the *tonB* region which are transcribed in the same direction as the *lacZ* gene (15). β-Galactosidase activity is present in these deletion strains and allows detection of these fusions as blue colonies on XG plates. The putative fusions were further screened for their Trp phenotype to remove from consideration any clones which bore *trp-lac* fusions. Seven out of 14 candidates were *trp*⁺ and apparent fusions to genes within the *tonB* region.

These fusions were crossed onto phage λp*trp-lac*W2Δ*nin*1, a *nin*1 deletion derivative of phage λp*trp-lac*W2 (4) which was constructed to accomodate more bacterial DNA. *trpA*⁺ plaques were selected on Ymel ΔA on Trp plates, purified, and retested for LacZ⁺ phenotype.

Preparation of transducing phage DNA. Phage f2 and its transducing phage derivatives were grown as described by Reznikoff et al. (20). The *tonB*⁻ *lacZ*⁺ fusion phages were found to grow best by using the modified PDS technique of Blattner et al. (8).

Phage preparations purified by two CsCl equilibrium density gradients were dialyzed against phage buffer. DNA was prepared by phenol extraction of these phages, followed by extensive dialysis against DNA buffer.

Preparation of plasmid DNAs. Plasmid DNAs were grown and prepared by the method of Blair et al. (7).

Cloning of restriction fragments into pRZ112. pRZ112 is a deletion variant of ColE1::Tn*5* constructed by Richard Jorgensen (R. Jorgensen and W. S. Reznikoff, manuscript in preparation). Tn*5* is a transposable element specifying neomycin resistance (6). Plasmid pRZ112 contains single *Hin*dIII, *Hpa*I, *Bgl*II, and *Eco*RI cleavage sites.

Plasmid and phage DNAs were cut with the desired restriction endonuclease, phenol extracted, ether extracted, and treated with T4 DNA ligase (23), and 0.1 to 0.4 μg of DNA was used to transform 0.2 ml of competent Ymel ΔA or W205, using a 45-s 37°C heat shock (13). Both Neo^r and Neo^r Chr^r colonies were screened for the presence of plasmids, using a modification of the "toothpick assay" of Barnes (3): clones to be screened for plasmids were amplified first in 2 ml of amplification medium and then treated as described above. All agarose gels, including those used to examine large DNA restriction fragments, were 1% agarose in the Tris-acetate-EDTA buffer described by Barnes (3).

The 2,720-bp *Pvu*II fragment was cloned by blunt-end ligation into the *Eco*RI site in pRZ112 after treatment of the *Eco*RI sticky ends with *Micrococcus luteus* DNA polymerase according to a modification of the Backman et al. (2) procedure by Hardies et al. (S. C. Hardies, R. K. Patient, R. D. Klein, F. Ho, W. S. Reznikoff, R. D. Wells, submitted for publication). This essentially involved heating the *Eco*RI-digested vector to 70°C to denature the sticky ends and kill the *Eco*RI enzyme. A 0.5-μg amount of this DNA was preincubated for 1 h at 15°C in ligase buffer plus four deoxyribonucleoside triphosphates (2 μM each) plus 1 μl of *M. luteus* DNA polymerase (150 U/ml) in a total volume of 16 μl. The *Pvu*II-digested DNA was then added along with T4 DNA ligase as described above.

Digestion of DNA with restriction endonucleases. A 3-μg amount of phage DNA or 1 μg of plasmid DNA was digested in *Hin*d buffer at 37°C in a final volume of 50 μl for all restriction endonucleases used in this study. The time of digestion and quantities of enzymes used varied with the enzyme preparation. *Hin*dII + III, *Eco*RI, and *Hin*dIII enzymes were generously provided by J. Gardner, S. Hardies, L. Maquat, and S. Rothstein. In some digests, *Hinc*II was used instead of *Hin*dII. All other enzymes were purchased from New England Biolabs.

Polyacrylamide gel analysis. Preparation of DNA samples has been described previously (12). DNA fragments were examined on 3.5% polyacryl-

amide slab gels (20 by 20 by 0.3 cm) containing 25% glycerol in TBE buffer (17). After electrophoresis at 110 V, the gels were stained in 10 μg of ethidium bromide per ml, destained in water, and photographed.

Molecular weight determinations. Double-stranded replicative form φX174 was digested with *Hin*cII, *Hpa*I, and *Hpa*II enzymes in separate digests, which were then combined and run beside the relevant phage DNA digests on polyacrylamide slab gels. The sizes of the restriction fragments were taken from Sanger et al. (22).

When larger molecular weight markers were needed (>2 × 10^6), *Eco*RI digestions of f2 were used. Molecular weights of these RI fragments came from Helling et al. (10). These digests were run on 1% agarose gels (data not shown).

RESULTS

Determination of the *Hin*dII and *Hin*dIII restriction maps. DNAs from two types of *tonB*⁻ transducing phages were analyzed by digestion with restriction endonucleases *Hin*dII and *Hin*dIII and electrophoresis of the digests on 3.5% polyacrylamide slab gels.

The first series of transducing phages was derived from a series of chromosomal *tonB* deletions in *E. coli* strain X7800 (*lacZ118*) which fused β-galactosidase to proteins within the *tonB* region (Fig. 1A). Three of these fusions have been shown to produce detectable levels of hybrid β-galactosidase proteins on sodium dodecyl sulfate gels (K. Postle, Ph.D. thesis, University of Wisconsin–Madison, 1978; K. Postle and W. S. Reznikoff, manuscript in preparation). In these *tonB*⁻ *lacZ*⁺ fusion phages, DNA is deleted from at least *attφ80* to *tonB* or beyond to *trp*. One phage, λp*trp-lac*K38, carries an apparent fusion of *lacZ* to *trpA*, resulting in a hybrid polypeptide with the activities of both genes (data not shown). All of the *tonB*⁻ *lac*⁺ fusion phages, of which λp*trp-lac*K37 is an example (Table 2), have the same *trp* end point and differ only in the extent of DNA deleted in creating the fusion.

The second series of transducing phages was derived from the λ-φ80 hybrid phage f2 (Fig. 1B). These phages differ from one another not

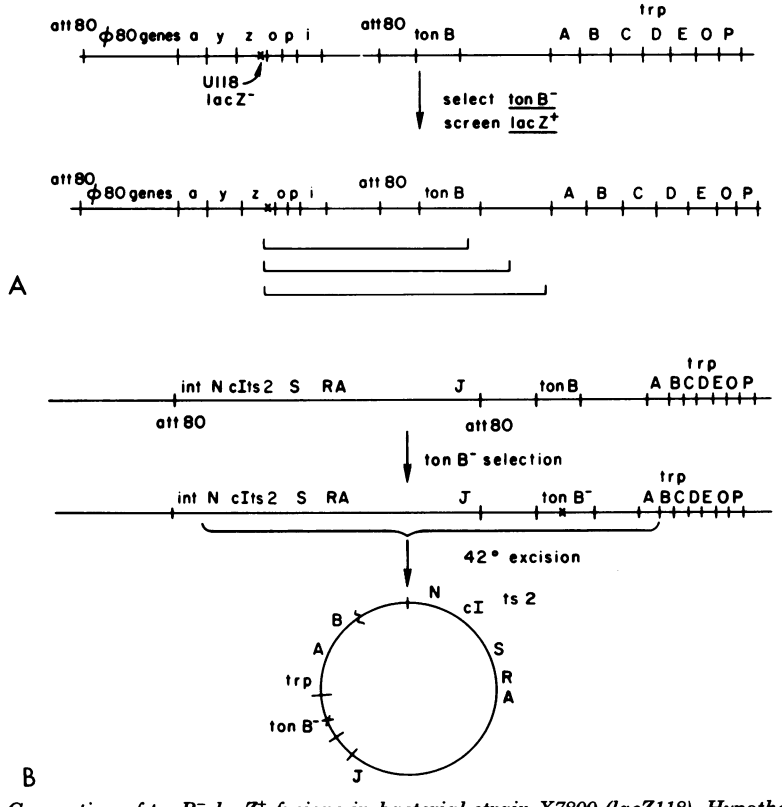

FIG. 1. *(A) Generation of tonB⁻ lacZ⁺ fusions in bacterial strain X7800 (lacZ118). Hypothetical deletions resulting in the tonB⁻ lacZ phenotype are diagrammed. lacZ end points are unknown, but all deletions result in detectable β-galactosidase activity. tonB region end points have been localized within various HindII+III restriction fragments. (B) Generation of f2tonB⁻ trpA⁺ transducing phages from the Ymel·f2 lysogen. A possible sequence of events resulting in phages such as f2#27 (Table 2) is diagrammed.*

TABLE 2. *Summary of restriction fragments present in HindII+III digests of tonB⁻ trpA⁺ transducing phage DNAs*

Phage	Fragment no.												
	7 (1,600)	6 (1,730)	8 (1,570)	28 (510)	20 (860)	27 (520)	43 (200)	41 (210)	17 (1,020)	16 (1,040)	25 (580)	23 (670)	4 (2,250)
f2≠27			+	+	+	+	+	+	+				
f2≠28	+	+	+	+	+	+	+	+	+				
f2trpA	+	+	+	+	+	+	+	+	+	+			
f2≠5				+	+	+	+	+	+	+	+		
f2≠32				+	+	+	+	+	+	+	+		
f2≠30	+			+	+	+	+	+	+	+	+		
f2≠22	+		+	+	+	+	+	+	+	+	+		
f2≠23	+		+	+	+	+	+	+	+	+	+		
f2≠29	+		+	+	+	+	+	+	+	+	+		
f2≠31	+		+	+	+	+	+	+	+	+	+		
f2≠17	+	+	+	+	+	+	+	+	+	+	+		
f2≠24	+	+	+	+	+	+	+	+	+	+	+		
f2≠18				+	+	+	+	+	+	+	+	+	+
13 4/6 5	+	+	+	+	+	+	+	+	+	+	+	+	+
λp*trp-lac*K37				+	+	+	+	+	+	+			
λp*trp-lac*K6						+	+	+	+	+			
λp*trp-lac*K48						+	+	+	+	+			
λp*trp-lac*K19							+	+	+	+			
λp*trp-lac*K29							+	+	+	+			
λp*trp-lac*K23								+	+	+			
λp*trp-lac*K38										+			

[a] Only restriction fragments from the *tonB-trp* region are summarized. The fragments are ordered left to right according to their map order. Plus (+) indicates that the restriction fragment was present in the *Hin*dII+III digest of phage DNA; a blank space indicates that the fragment was missing. Numbers within parentheses are lengths in base pairs of the numbered fragments under which they appear. Phage 13 4/6 5 described by Jones and Reznikoff (12) carries the wild-type *tonB* region and the entire *trp* operon.

only in the extent of the *trp* operon they carry, but also in the types of *tonB* mutations present. There are three classes of *tonB* mutations which can be discerned (Table 2): (i) those mutations which do not detectably alter any restriction fragments, such as in phages f2≠28, f2≠24, and f2≠17; (ii) those mutations which alter only fragment 6 and may be either deletions or insertions, such as in phages f2≠23, f2≠22, f2≠29, and f2≠31; (iii) those mutations which are obviously deletions by the criteria that they remove two or more contiguous restriction fragments, such as in phages f2≠27, f2≠5, f2≠30, f2≠32, and f2≠18.

Previous *Hin*dII+III restriction analysis of f2*tonB*⁺ *trp*⁺ phages had identified 13 restriction fragments unique to the region extending from *attφ80* through *tonB* and through the *trp* operon (12). These are numbered according to their order in the *Hin*dII+III restriction pattern of the *tonB*⁺ *trp*⁺ phage 13 4/6 5. The lengths in base pairs of these fragments are as follows: 4, 2,250; 6, 1,730; 7, 1,600; 8, 1,570; 16, 1,040; 17, 1,020; 20, 860; 23, 670; 25, 580; 27, 520; 28, 510; 41, 210; 43, 200. The molecular weights from Jones and Reznikoff (12) have been updated for this paper, using the more accurate φX174 DNA restriction fragments as markers.

Representative *Hin*dII+III digests are shown in Fig. 2. The absence of fragments 25, 23, and 4 from a digest of the *tonB*⁺ *trpA*⁺ *trpB*⁻ phage f2*trpA* indicated that they are *trp* fragments (Table 2). Likewise, the digest of f2≠28 assigned fragment 16 to the *trp* operon. Analysis of *tonB*⁻ *trp*⁺ phage digests such as f2≠23 implicated fragment 6 in the *tonB* mutations. The large deletion of fragments 6, 7, and 8 in f2≠32 allowed assignment of fragments 7 and 8 to a cluster along with fragment 6. Finally, the fusion phages provided a valuable deletion analysis of the region, which permitted the construction of the *Hin*dII+III map (Fig. 3) with four ambiguities: the relative orders of fragments 23 and 4, fragments 20 and 28, fragments 41 and 43, and fragments 6, 7, and 8.

(i) Relative order of fragments 23 and 4: Fragments 4, 16, 20, 23, 25, and 27 were absent when phage 13 4/6 5 DNA was digested with *Hin*dII instead of *Hin*dII+III. Three new fragments appeared of lengths 2,900, 1,400, and 1,600 bp. On the basis of their molecular weights and the data in Table 2, these three new fragments were identified as fusions of fragments 4 and 23, 16 and 25, and 20 and 27, each of which contains a single *Hin*dIII site at the junction of the two *Hin*dII+III fragments. This information allowed the construction of a *Hin*dIII map of the *tonB-trp* region. Table 2 predicts the presence of a *Hin*dIII fragment consisting of fragments 25 and either 4 or 23. *Hin*dIII digest of f2, f2*trpA*, and

13 4/6 5 DNAs reveals a unique ~1,300-bp fragment in 13 4/6 5 which, on the basis of its size, must consist of fragments 25 and 23. Therefore, the order is 25-23-4.

(ii) Relative order of fragments 20 and 28: Knowledge of the *Hin*dIII site separating fragments 20 and 27, together with the *Hin*dII+III digest of fusion phage λp*trp-lac*K6 (Table 2), suggests the correct order. The λp*trp-lac*K6 digest lacks fragments 20 and 28, but has fragment 27. Since fragment 20 must be next to fragment 27, the order must be 28-20-27.

(iii) Relative order of fragments 17 and 41: The nucleotide sequence of the *trpA-trpB* intercistronic region determined by Platt and Yanofsky (18) predicts an *Hpa*I site in *trpB* DNA, 66 bp prior to the *trpB* translation stop codon. *Hpa*I cleaves sequences which are a subset of those cleaved by *Hin*dII (21). *Hpa*I + *Hin*dIII double digests of various f2-derived phages indicate that the only *Hpa*I site within the *trp* structural genes is located between fragments 17 and 16. Since f2 phages such as f2≠27 (Table 2) which carry *trp* DNA up to but not including fragment 16 are *trpA*$^+$, our data are consistent with the *Hpa*I site predicted by the data of Platt and Yanofsky that is located between fragments 16 and 17. Fusion phage λp*trp-lac*K38 is *trpA*$^+$ (as are all of the fusion phages) but lacks both fragments 41 (210 bp) and 17 (1,020 bp). Since fragment 41 is only 210 bp long and since λp*trp-lac*K38 must contain at least 800 bp beyond the

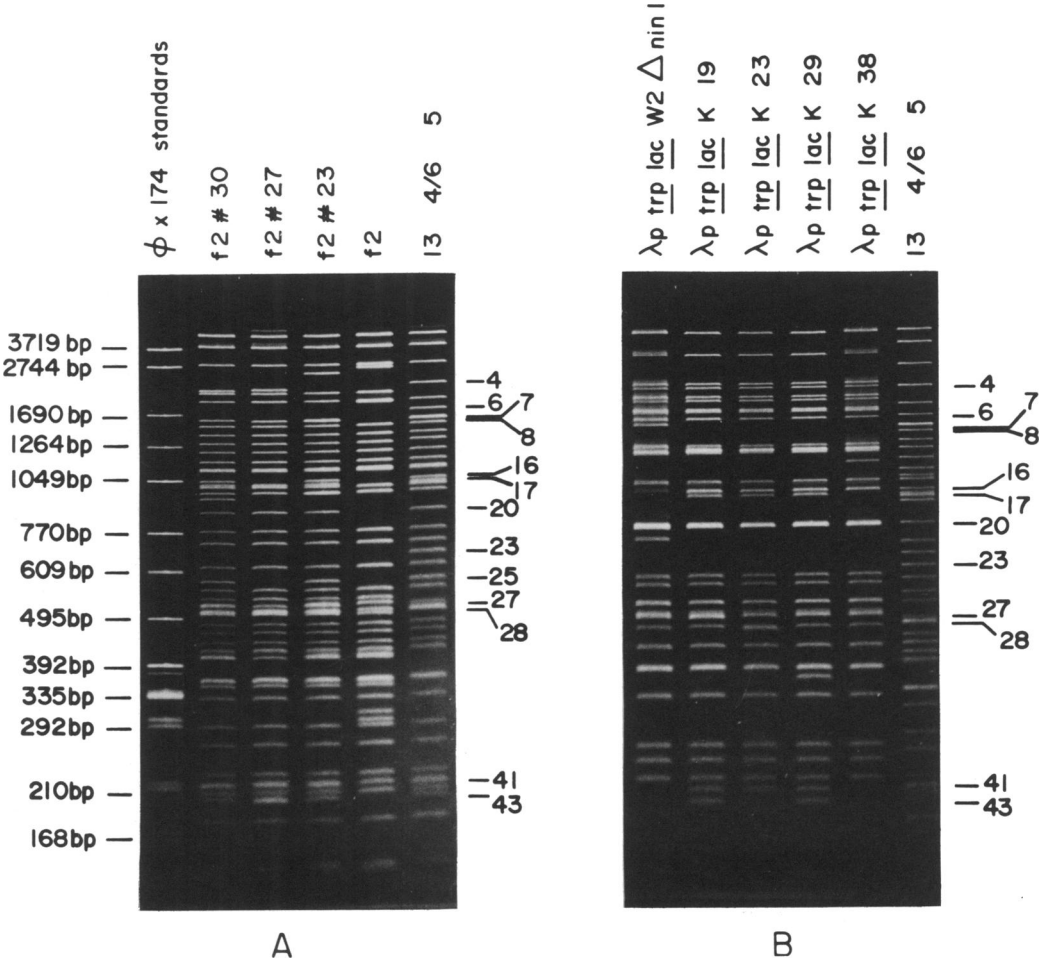

FIG. 2. *HindII+III digests of f2-derived transducing phage DNAs (A) and λptrp-lac W2-derived fusion phage DNAs (B). Numbers on the right-hand side of each figure correspond to the tonB-trp restriction fragments from phage 13 4/6 5 mentioned in the text and shown in Fig. 3. φX174 standards consist of a mixture of three separate restriction digests (HpaI, HpaII, and HincII) as indicated in the text. 3.5% polyacrylamide-25% glycerol slab gels were run for 12 h at 110 V in TBE buffer (16).*

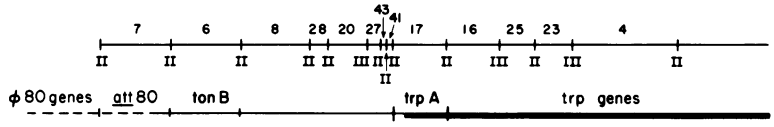

FIG. 3. *HindII and HindIII restriction maps of the tonB-trp region. II indicates HindII restriction sites. III indicates HindIII restriction sites. Correlation of the physical map with the genetic map is shown. The elements on the genetic map have been localized to the restriction fragments under which they appear.*

tonB side of the *Hpa*I site to accommodate the *trpA* gene, the absence of fragment 41 from a *Hin*dII+III digest of λp*trp-lac*K38 suggests that the order is 41-17-16.

(iv) Relative order of fragments 6, 7, and 8: *Hin*dII+III digests of the transducing phages did not readily provide the order of fragments 6, 7, and 8. However, 6 was known to be the internal band on the basis of f2#27 and f2#30 digests, which deleted 6 and 7 or 6 and 8, respectively (Table 2). To aid in the restriction analysis, the 10,000-bp *Hin*dIII fragment was inserted into the single *Hin*dIII site in pRZ112, a neomycin-resistant deletion derivative of ColE1::Tn*5*, to yield the hybrid plasmid pRZ510 (Fig. 4).

The fact that *Bgl*I cuts in fragment 8, but not in 6 or 7, was used to order these fragments within the plasmid. *Hin*dIII + *Bgl*I digests of parental plasmid pRZ112 and *tonB*$^+$ plasmid pRZ510 show that only two new fragments arise from the cleavage of the cloned 10,000-bp *Hin*dIII fragment by *Bgl*I (Fig. 5A). The lengths of these two fragments which must have been generated by the *Bgl*I cut in fragment 8 are 2,500 and 7,300 bp. (The 7,300-bp fragment comigrates with the largest fragment in the digest.) If the fragment order were 8-6-7-28-20, the *Bgl*I + *Hin*dIII fragment containing 28 and 20 should have been between 3,700 and 5,270 bp. If the fragment order were 7-6-8-28-20, the *Bgl*I + *Hin*dIII fragment containing 28 and 20 should be between 1,370 and 2,940 bp. Clearly, the 2,500-bp *Bgl*I + *Hin*dIII fragment which is observed is most consistent with the order 7-6-8-28-20.

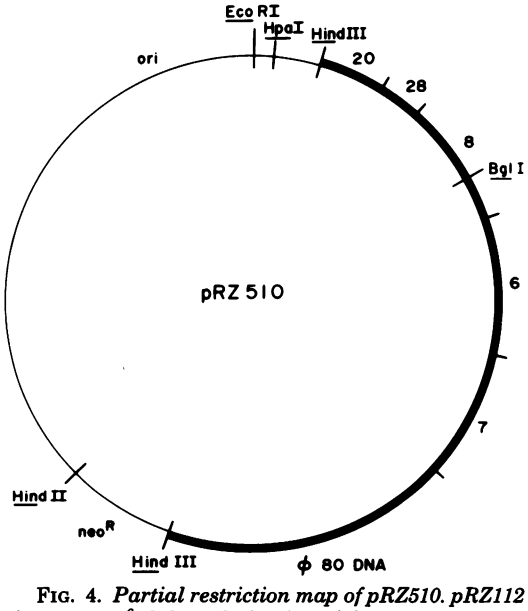

FIG. 4. *Partial restriction map of pRZ510. pRZ112 is a 6 × 10^6-dalton derivative of ColE1 onto which Tn5 (specifying neomycin resistance) has been transposed. The hybrid plasmid pRZ510 containing the inserted 10,000-bp HindIII fragment is diagrammed here. The heavy line corresponds to the inserted HindIII fragment. The numbered fragments on that line correspond to the HindII fragments contained within the HindIII fragment. The BglI site in HindII fragment 8 is indicated.*

Other restriction sites in the *tonB* region. Additional restriction cleavage sites in the *tonB* region are shown in Fig. 6. The *Hpa*I and *Sal*I sites were obtained by double and triple digest of f2-derived phage DNAs with *Hin*dIII, *Hpa*II, and *Sal*I. These were confirmed with similar digests of pRZ510 and pRZ429, a hybrid plasmid containing a *Hin*dIII fragment consisting of *Hin*dII+III fragments 27, 43, 41, 17, and 16. *Pvu*II, *Bgl*I, *Bgl*II, *Pst*I, and *Ava*I sites were mapped in pRZ526, a hybrid plasmid carrying an *Hpa*I fragment consisting of *Hin*dII+III fragments 7, 6, and 8 inserted into the *Hpa*I site of pRZ112.

Location of *tonB*. A class of *tonB*$^-$ *trpA*$^+$ f2 phages exists which is altered in only the 1,730-bp fragment 6 (Table 2, f2#22, f2#23, f2#29, and f2#31). The existence of this class suggests that *tonB* is at least partially contained within the 1,730-bp fragment. To locate *tonB* more precisely, we have begun to construct hybrid plasmids containing various restriction fragments from the *tonB* region (Table 3 and Fig. 5B, lanes 1 to 5).

These hybrid plasmids were selected by their ability to complement chromosomal *tonB* deletions with respect to chromium resistance. Chr^r candidates were also tested for sensitivity to φ80*vir* and colicin V+B. *tonB*$^+$ bacteria are resistant to 100 μM chromium, whereas *tonB* bacteria are sensitive to this metal. All of the *tonB*$^+$

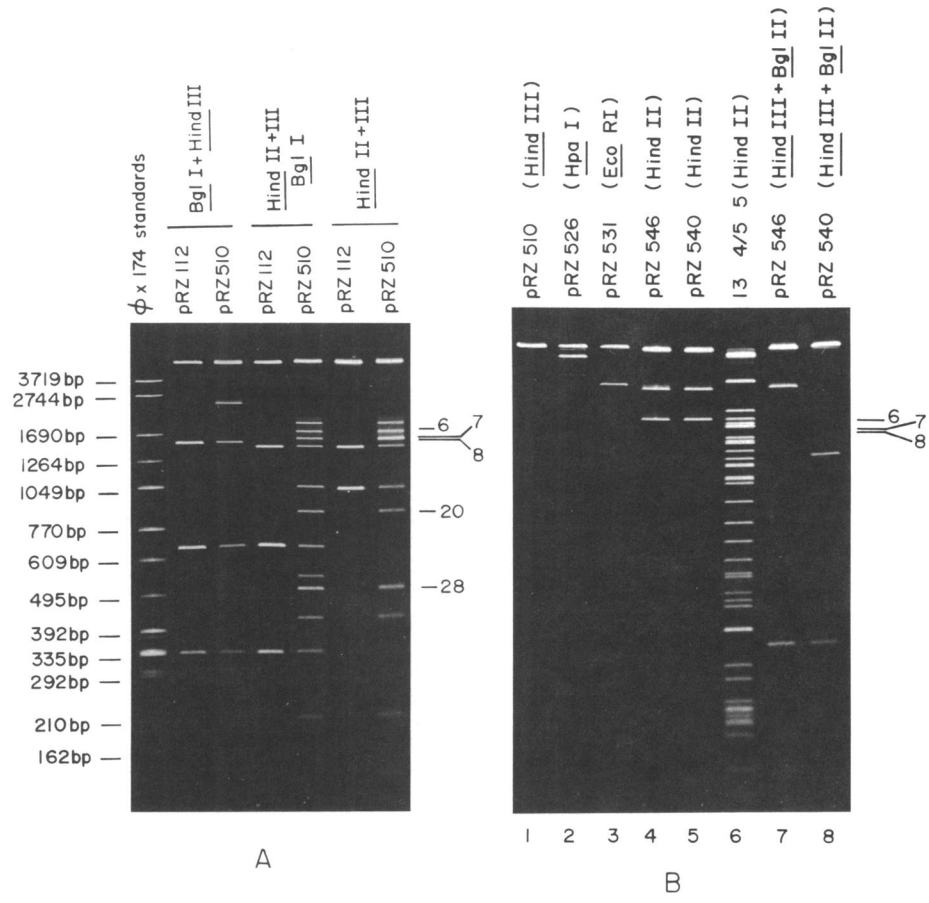

FIG. 5. *Restriction endonuclease digests of tonB⁺ hybrid plasmids. (A) Comparative digests of pRZ112 (parental plasmid) and pRZ510 (hybrid plasmid carrying the tonB⁺ 10,000-bp HindIII fragment diagrammed in Fig.4). The BglI + HindIII digest patterns demonstrate a unique BglI site in the 10,000-bp HindIII fragment. Comparison of the HindII+III + BglI digest with the HindII+III digest confirms this analysis and demonstrates that the BglI site is in HindIII fragment 8. (B) Restriction digests of hybrid plasmids carrying cloned tonB⁺ restriction fragments. Each lane is labeled with the plasmid name (Table 3) and the restriction enzyme used for digestion. The numbers to the right of the figure show positions of bands 6, 7, and 8 in the HindII digest of 13 4/6 5 (lane 6). Lanes 1 to 5 demonstrate that successively smaller restriction fragments have been cloned. The HindIII band in lane 1 is a doublet consisting of pRZ112 plus the cloned HindIII fragment. The cloned PvuII fragment can be excised from pRZ531 by using EcoRI as explained in the text. The smallest fragments in pRZ546 and pRZ540 (lanes 4 and 5) are both the cloned 1,730-bp HindII fragment 6 as shown by comparison with the HindII digest of 13 4/6 5 (lane 6). The HindIII + BglII double digest of pRZ546 and pRZ540 (lanes 7 and 8) indicate that the 1,730-bp fragment has been cloned in both orientations. The parental plasmid pRZ112 contains one BglII site and one HindIII site. The inserted 1,730-bp fragment contains one BglII site 170 bp from the end of the fragment (Fig. 6).*

restriction fragments cloned contain the 1,730-bp fragment 6 (Table 3). To aid with the initial restriction mapping, we have also cloned the *Hin*dIII fragment containg *Hin*dII+III fragments 27, 43, 41, 17, and 16 on the basis of *trpA*⁺ expression (pRZ429). The 1,730-bp fragment 6 has been cloned in both orientations relative to the vector pRZ112 (Fig. 5B lanes 7 and 8). When transformed into a bacterial strain carrying a total chromosomal *tonB* deletion (W205), both plasmids (pRZ546 and pRZ540) confer chromium resistance, φ80 sensitivity, and colicin V+B sensitivity on the transformants.

DISCUSSION

Deletion analysis of *Hin*dII+III restriction fragments from various *tonB⁻ trpA⁺* transducing phage DNAs has allowed us to order the *Hin*dII

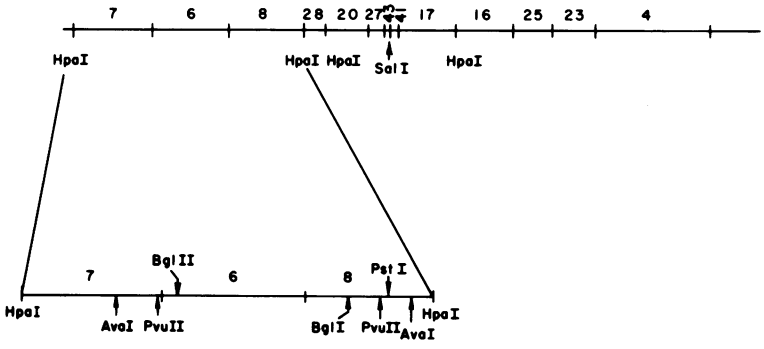

FIG. 6. *Other restriction endonucleases sites in the tonB region. The numbered HindII+III fragments are as in Fig. 3. The expanded map section corresponds to the cloned HpaI fragment of pRZ526. Restriction enzymes AvaI, PvuII, BglII, BglI, and PstI have been mapped only within this fragment. There are no EcoRI, BamHI, KpnI, XbaI, or SmaI sites within the HpaI fragment.*

TABLE 3. *pRZ112 hybrid plasmids*[a]

Plasmid	Inserted DNA			Selected phenotype
	Type of fragment	Size (bp)	*Hin*dII+III fragments	
pRZ429	*Hin*dIII	2,990	27, 43, 41, 17, 16	TrpA$^+$
pRZ510	*Hin*dIII	10,000	7, 6, 8, 28, 20, additional ϕ80 DNA	TonB$^+$
pRZ526	*Hpa*I	4,900	7, 6, 8	TonB$^+$
pRZ531	*Pvu*II	2,760	6, partial 7, partial 8	TonB$^+$
pRZ540	*Hin*dII	1,730[b]	6	TonB$^+$
pRZ546	*Hin*dII	1,730	6	TonB$^+$

[a] Construction of hybrid plasmids is presented. *Hin*dIII, *Hpa*I, *Pvu*II, and *Hin*dII fragments were inserted into *Hin*dIII, *Hpa*I, *Eco*RI, and *Hpa*I sites on pRZ112, respectively.

[b] 1,730-bp insert is in opposite orientation from pRZ540.

and *Hin*dIII fragments in the *tonB-trp* region of the *E. coli* genome. The observation that several of these transducing phages are altered only in fragment 6 suggests that this 1,730-bp fragment contains at least part of the *tonB* gene. The localization of *tonB* has been confirmed by the construction of hybrid ColE1 plasmids into which the 1,730-bp fragment has been inserted in both orientations. These hybrid plasmids complement a total chromosomal deletion of the *tonB* gene with respect to chromium resistance, ϕ80 sensitivity, and colicin V+B sensitivity, suggesting that the *tonB* is entirely contained within the *Hin*dII fragment.

The *Hin*dII+III restriction map also provides an estimate of physical distances within the *attϕ80-tonB-trpA* genetic map. The location of *trpA* is well defined since it must be entirely contained within the 1,020-bp fragment 17. The location of the *Hpa* site separating fragments 16 and 17 in *trpB* (18), together with the known size of the *trpA* polypeptide (24), indicates that the *tonB* side of fragment 17 is only 145 to 155 bp from the end of *trpA*. The map location of *attϕ80* defined by the junction of phage and bacterial DNA is uncertain. If we choose a nominal molecular weight of approximately 36,000 for the *tonB* polypeptide (K. Postle and W. S. Reznikoff, manuscript in preparation) and assume that *tonB* is entirely contained within the 1,730 *Hin*dII fragment 6, we obtain a maximum estimate for the distance from *attϕ80* to *tonB* of 2,500 bp. However, we can place no lower limit upon this distance. Thus, *tonB* is at least 4,020 bp from *trpA*, and our estimate for the size of the entire region from *attϕ80* to *trpA* is 6,550 $\pm$ 800 bp.

As mentioned above, the only mutations which characterize the *tonB* region are those in *tonB* itself. The following properties of the *tonB*$^-$ *lacZ*$^+$ fusion phages imply the existence of other genes coding for polypetides between *tonB* and the end of *trpA*. (i) The *tonB* deletions which created the *lacZ*$^+$ fusions in X7800 (*lacZ118*) all remove and extend beyond fragment 6. (ii) These fusions all have detectable levels of β-galactosidase (data not shown). We conclude that the region between *tonB* and *trpA* codes for at least one other polypeptide and that this polypeptide is transcribed in the same direction as the *trp* operon.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant GM 19670 from the National Institute of General Medical Sciences. W. S. R. was supported in part by Public Health Service Career Development Award GM 30970 from the National Institute of General Medical Science and is a recipient of the Harry and Evelyn Steenbock Career Development Award.

We thank Kevin Bertrand for critical reading of the manuscript and for pointing out the possible existence of an *Hpa*I site in *trpB*.

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