Genetic Structure and DNA Sequences at Junctions Involved in the Rearrangements of *Bacillus subtilis* Strains Carrying the *trpE26* Mutation

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ABSTRACT

Studies on the region upstream to ribosomal operon *rrnD* of *Bacillus subtilis* led to the characterization of two of the four chromosomal junctions involved in the rearrangements (a translocation and an inversion) of the strains carrying the *trpE26* mutation. Genetic analysis, by integrative mapping, showed linkage of *rrnD* to *cysB* and *hisA* (both on segment A) in the *trpE26*-type strains. Physical analysis showed that the region upstream to *rrnD* is now linked to the *trpE-ilvA* chromosome segment as demonstrated by analyzing restriction site-polymorphism between 168 and *trpE26*-type strains. Similar experiments confirmed the previous genetic data on linkage in these areas in strains carrying novel rearrangements derived from the *trpE26*-type strains: stable merodiploids and inversions. The nucleotide sequence of the area 5′ to *rrnD* in both types of strains (168 and *trpE26*), the region downstream of the *citG* gene and the region carrying the *trpE26* mutation (made available to us by D. Henner) provided evidence for the molecular basis of the differences in structure, allowed the identification of the break points and revealed the presence of a polypurine region upstream to *rrnD* as seen in other systems in *B. subtilis*. No extensive homology was found between pairs of junctions so far sequenced. The models proposed by C. Anagnostopoulos for the role of DNA sequences of intrachromosomal homology involved in the transfer of the *trpE26* mutation and the formation of novel arrangements require therefore reevaluation.

STUDIES on genetic rearrangements are likely to provide information on the structure, function and evolution of bacterial genomes as well as mechanisms of recombination. In the genetically well studied enteric bacteria *Escherichia coli* and *Salmonella typhimurium*, several types of rearrangements are known to occur or to be readily induced in the laboratory. In *Bacillus subtilis* however, chromosomal rearrangements have so far been the subject of a limited number of investigations. The best known case is the "*trpE26* mutation." The original mutant carrying it, 166, is one of the biochemical mutants isolated by BURKHOLDER and GILES (1947) after X-ray irradiation of spores from the *B. subtilis* Marburg strain. The sole phenotypic trait of 166 is a tryptophan requirement due to the splitting of the *trpE* locus; the nature of the mutation is however much more complex. Strain 166 possesses in fact two extensive adjacent chromosomal rearrangements: a translocation of the *trpE-ilvA* segment comprising 4% of the genome and an inversion involving 36% of the upper part of the chromosome including the origin of replication from *cysB-0-tre* (Figure 1 and ANAGNOSTOPOULOS 1977). One of the endpoints of the translocated segment is inside the *trpE* gene. Another mutant they isolated was 168T containing only the *trpC2* mutation and is now the most commonly used *B. subtilis* strain. What was surprising is that in reciprocal genetic crosses between 168-type and 166 strains, and in the crosses of the progeny, novel rearrangements occurred: tandem and nontandem duplications, deletions and inversions. For example, the two original rearrangements of the 166 strain (translocation and inversion) can be transferred simultaneously to a 168-type strain by transformation or transduction. The strains carrying these rearrangements in a 168-type genetic background are called *trpE26*-type strains. The properties of the system were extensively studied by ANAGNOSTOPOULOS and co-workers, who characterized the rearrangements genetically and established the genetic maps of these strains (AUDIT and ANAGNOSTOPOULOS 1972, 1973, 1975; TROWSDALE and ANAGNOSTOPOULOS 1975, 1976; SCHNEIDER, GAISNE and ANAGNOSTOPOULOS 1982; O'SULLIVAN and ANAGNOSTOPOULOS 1982; SAMMONS and ANAGNOSTOPOULOS 1982; for review see ANAGNOSTOPOULOS 1990). It was postulated that the transmission of these rearrangements as well as the formation of novel rearrangements except for the tandem duplications takes place by recombination at DNA sequences of intrachromosomal homology (ANAGNOSTOPOULOS 1977, 1990). Three sets of sequences labeled 1, 2 and 3 were proposed and their locations were assigned to the endpoints of the rearrangements. The genetic maps of the 168- and *trpE26*-type strains

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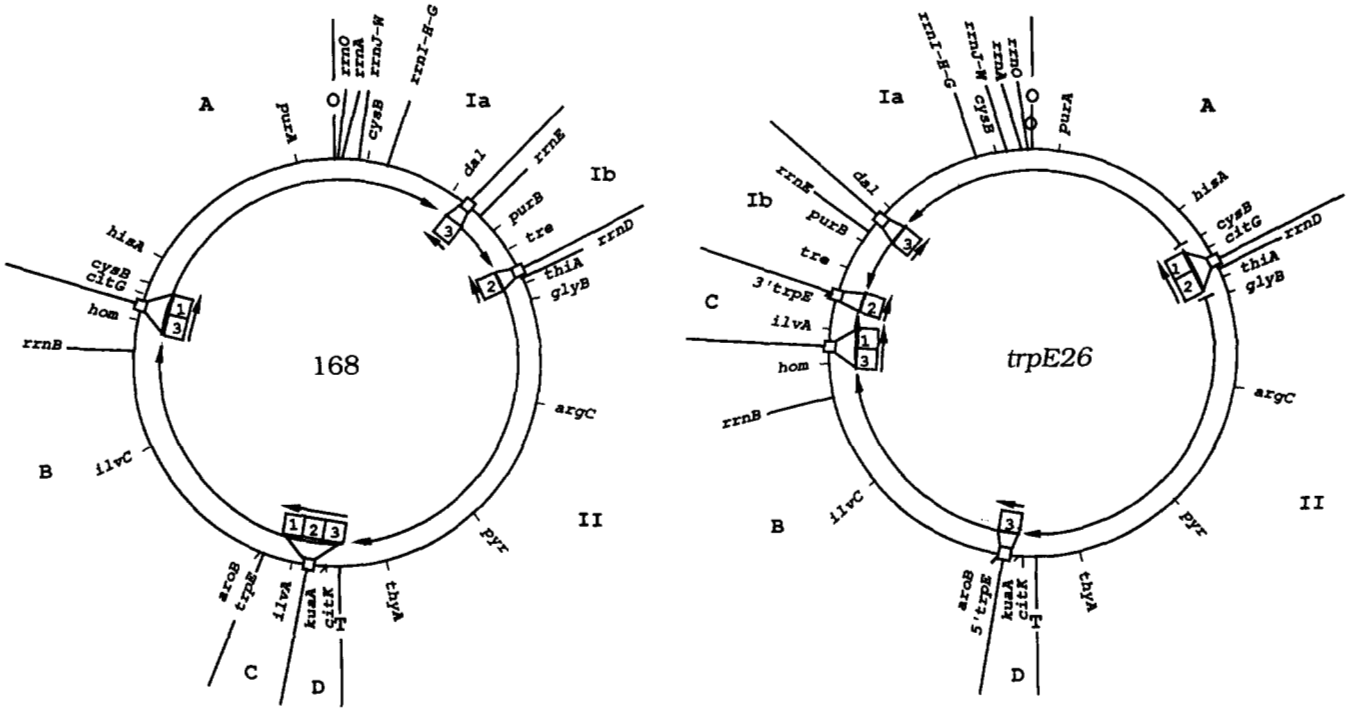

FIGURE 1.—Abridged genetic maps of 168- and *trpE26*-type strains of *B. subtilis* as reported by SCHNEIDER, GAISNE and ANAGNOSTOPOULOS (1982). The location of *rrn* operons are as reported by JARVIS *et al.* (1988). The capital (A–D) and Roman numerals (Ia, Ib and II) designate regions of the chromosome inside which no rearrangements have so far been observed. O and T are the origin and terminus of replication. The arrows indicate orientation of the regions based on the convention of reading the 168 strain map clockwise from the origin. The open rectangles at the junctions correspond to postulated sequences of intrachromosomal homology of three kinds (1, 2 and 3). The location of the split *trpE* gene was added to the map of *trpE26*.

are shown in Figure 1. The position and extent of the translocation and the inversion are clearly indicated. The drawings are taken from SCHNEIDER, GAISNE and ANAGNOSTOPOULOS (1982); the figure also shows the putative homologous sites. We added on to the maps the *rrn* operons which we have recently mapped (JARVIS *et al.* 1988).

In the course of our studies on the *rrn* operons of *B. subtilis* we became interested in the *trpE26* rearrangements. These redundant gene clusters have a fair possibility to be involved in homologous recombination events leading to rearrangements. Ribosomal RNA (*rrn*) genes have already been found at sites of rearrangements in *E. coli* and *S. typhimurium* (HILL and HARNISH 1982; HILL, HARVEY and GRAY 1990) as well as in *B. subtilis* (WIDOM *et al.* 1988). In *B. subtilis* several observations were in favor of involvement of *rrn* operons in the *trpE26* rearrangements: (1) the multiplicity of these operons and their unique tandem arrangements in certain regions (WIDOM *et al.* 1988; JARVIS *et al.* 1988); (2) the map positions of *rrnD* and *rrnE* in areas where rearrangements occurred; and (3) we found differences in hybridization patterns with *Hind*III digests: a new band of 3.3 kb appeared with DNA from *trpE26*-type strains (GOTTLIEB, LAFAUCI and RUDNER 1985).

In this paper we show that restriction pattern differences were due to a disruption of an area upstream to the *rrnD* operon and not to its coding region. Moreover, *rrn* operons do not serve as homologous sequences for recombination events in the *trpE26* system as they do in the enteric bacteria. Using integrative plasmids into the *rrn* operon it was possible to isolate, sequence and map this upstream area from both the normal 168-type and the rearranged *trpE26*-type strains. The areas were identified to contain the Ib-II and A-II junctions of 168- and *trpE26*-type strains, respectively (Figure 1). Comparisons were made to the following sequenced junctions: (1) the A-B junction downstream of the *citG* gene in a 168-type strain; (2) the B-C junction in a 168-type strain which is within the intact *trpE* gene (BAND, SHIMOTSU and HENNER 1984; HENNER, BAND and SHIMOTSU 1984) and (3) the Ib-C junction of a *trpE26*-type strain (L. BAND and D. T. HENNER, unpublished results, mentioned in BAND, SHIMOTSU and HENNER 1984). This analysis allowed us to locate the exact endpoints of the junctions. No sequences of extensive intrachromosomal homology were found. The results are discussed below in relation both to the mechanism of X-ray induced rearrangements in strain 166 and to the proposed models for the formation of subsequent novel rearrangements in genetic crosses using this strain or its progeny.

MATERIALS AND METHODS

Bacterial strains and plasmids: The *B. subtilis* strains and plasmids used in this study are described in Table 1.

TABLE 1

Strains and plasmids used in this study

Name and number	Genotype	*rrn*[a]	Origin and remarks
I. Parental strains			
NCTC3610	Prototroph	10	A. SONESHEIN
168T	*trpC2*	10	K. BOTT
166	*trpE26*	10	BGSC[b]
GSY1269	*trpE26 ilvC1*	9	K. BOTT
GSY1127	*hisH2 ilvC1/ilvC*$^+$	9	C. ANAGNOSTOPOULOS
GSY1835	*trpE30*	9	C. ANAGNOSTOPOULOS
SB25	*trpC2 hisH2*	10	D. DUBNAU
BD111	*trpC2 cysB3 thrA5*	ND	D. DUBNAU
BD115	*trpC2 hisA1 thrA5*	ND	D. DUBNAU
BD170	*trpC2 thrA5*	9	D. DUBNAU
RR23	*trpE26 cysB3*	9	BD111 → GSY1269[c]
RR33	*trpE26 hisA1*	9	BD115 → GSY1269[c]
RR36	*trpE26 thrA5*	9	BD170 → GSY1269[c]
Kit 1 to kit 9	Mapping recipients	ND	D. DUBNAU
II. Integrants[d]			
168T-18	pGR102 *trpC2* Cm^r	9	E. JARVIS *et al.* (1988)
SB25-39	pGR110 *trpC2 hisH2* Cm^r	8	This study
BD170-10	pGR111 *trpC2 thrA-5* Cm^r	8	This study
166-96	pGR102 *trpE26* Cm^r	9	This study
166-99	pGR111 *trpE26* Cm^r	9	This study
GSY1269-25	pGR110 *trpE26 ilvC1* Cm^r	8	This study
GSY1269-28	pGR110 *trpE26 IlV*$^+$ Cm^r	8	This study
III. Plasmids			
pWR305[e]	Tc^r Cm^r *rrnD* (168)		This study
pJR421[f]	Tc^r Cm^r *rrnD* (166)		This study
pJR412[g]	Am^r Cm^r *rrnD* (166)		This study
p*trp*1A65	Am^r *trpE* (166)		D. HENNER
pAMM2	Am^r *citG-gerA* (168)		A. MOIR

[a] Number of intact *rrn* operons. The strains with 9 operons have a natural deletion of *rrnG* (WIDOM *et al.* 1988). Integrants with a Cm^r marker disrupt *rrnD* making the strains contain either 9 or 8 intact *rrn* operons. SB25-39 had a spontaneous deletion of *rrnG*. ND, not determined.

[b] Bacillus Genetic Stock Center, Columbus, Ohio.

[c] Strains obtained by transformation. The arrow points to the recipient strain.

[d] The integrated plasmids contain ribosomal sequences from within the transcriptional units for *16S* and *23S* have been described in detail in LAFAUCI *et al.* (1986) and Jarvis *et al.* (1988).

[e-g] Plasmids rescued from self-ligated *BclI* digests of DNA from strains: SB25-39, GSY1269-25, and 166-96, respectively.

The parental strains were used either as donors or recipients in transformation and transduction crosses. Indicated are the number of *rrn* operons in each strain. Integrant strains were obtained by transformation with one of six integrable plasmids all of which are derivatives of the parent pJH101 (FERRARI *et al.* 1983) as reported by us previously (LAFAUCI *et al.* 1986 and JARVIS *et al.* 1988) or by using donor DNA with an integrated plasmid. Plasmids pGR102, pWR103, pGR110 and pGR111 contain a 1.2-, 1.0-, 0.5- and 1.5-kb fragment of *B. subtilis* 16S-2tRNA genes-23S, 16S-23S, 16S and 23S genes, respectively. Plasmid pWR112 contains a *lacZ* gene fused to 16S-23S genes in the vector pDEB1 (WIDOM 1988). We have designated the various *B. subtilis* transformants as the parent strain dash (–) clone number and the specific plasmid integrated into an *rrn* operon (JARVIS *et al.* 1988).

Culture conditions, transformation and transduction: All genetic methods for *B. subtilis* and *E. coli* as well as the integrative mapping procedure were described previously (LAFAUCI *et al.* 1986; JARVIS *et al.* 1988). Cm^r integrants were selected on LB plates containing 10 μg of chloramphenicol (Sigma) per ml. In order to be able to map genes near a rearranged junction, both donor and recipient strains must have the same genetic orientation (Figure 1). Suitable recipients for PBS-1 mediated transductional mapping with the genetic background of *trpE26*-type strains were constructed by congression in transformation using high DNA concentrations (10–20 μg/ml). The *cysB3*, *hisA1* and *thrA5* markers which are normally localized at map positions 300, 305 and 270, respectively (Figure 1; PIGGOT and HOCH 1985), were thus transferred to GSY1269 through the selection of Ilv$^+$ transformants on minimal plates containing 50 μg per ml of the appropriate amino acid; *i.e.*, cysteine, histidine or threonine. These transformants were replica plated on the appropriate plates to screen for the transferred genetic marker. The constructed recipients are listed in Table 1.

DNA manipulations: Chromosomal DNA was prepared as described previously (LAFAUCI *et al.* 1986; JARVIS *et al.* 1988). Plasmid DNA was purified from *E. coli* cultures by the procedure of TANAKA and WEISBLUM (1975). Rapid plasmid and M13 RF DNA isolation was done by the alkaline lysis procedure (BIRNBOIM and DOLY 1979). Single stranded M13 phage DNA was isolated by the method of MESSING (1983). For isolation of small amounts of chromosomal DNA 5 ml overnight cultures of *B. subtilis* were used. The cells were resuspended in 0.2 ml of SET buffer (20% sucrose, 50 mM EDTA and 50 mM Tris, pH 7.6) containing 5 mg/ml lysozyme and 1 mg/ml RNase, vortexed well and incubated at 37° for 15 min. To the lysate 0.4 ml of 1% SDS was added and mixed well by inversion followed by a 10-min incubation at 60°. An equal volume (0.6 ml) of a 1:1 mixture

containing 80% phenol: chloroform/isoamyl alcohol (24/1) was added, vortexed and centrifuged for 5 min. The aqueous layer was removed, and reextracted with chloroform/isoamyl alcohol. Nucleic acids were precipitated with an equal volume of isopropanol after adjustment to 0.3 M Na acetate, and centrifuged for 10 min. The pellet was washed twice with 70% ethanol, dried for 15 min. in a Speed Vac (Savant) and dissolved in 50–100 μl of H_2O. Typical recoveries were 25–50 μg of chromosomal DNA per sample. Chromosomal DNA was digested for Southern hybridizations as described previously (LaFauci *et al.* 1986). For plasmid rescue, DNA from integrant strains was digested with *Bcl*I, self-ligated at low (2 μg/ml) DNA concentration and transformed into *E. coli* HB101 as described previously (Widom *et al.* 1988); see Table 1 for the list of rescued plasmids and their parental strains.

DNA labeling, Southern blotting and sequencing: Plasmid DNA, isolated DNA fragments, or lambda DNA were labeled with ^{32}P using the random primed synthesis kit as directed by the supplier (Boehringer Mannheim Biochemicals). DNA digested with *Bcl*I, *Hin*dIII or *Eco*RI was transferred to nitrocellulose filters from 0.75% agarose gels and hybridized according to the method of Southern (1975) as described previously (LaFauci *et al.* 1986; Jarvis *et al.* 1988). DNA sequencing was carried out by the dideoxy-chain termination method (Sanger, Nicklen and Coulson 1977) using a sequenase kit of Boehringer Mannheim. To sequence the region upstream to *rrnD* from a 168-type strain, a 2.2 kb *Hin*dIII fragment containing the 5′ end of 16S rDNA (see Figure 3b) from rescued plasmid pWR305 was subcloned into a similarly cut M13mp18 vector (for unexplained reasons no clones could be recovered from M13mp19). To sequence the 5′ region upstream to *rrnD* from a *trpE26*-type strain the double-stranded rescued plasmid template pJR421 was used directly. Sequences of both upstream regions were determined with the aid of two oligonucleotide primers synthesized on an Applied Biosystems model 380A. The first primer (5′GCTCGATTGCATGTAT3′) was complementary to bases 67–51 of the 16S gene (Green *et al.* 1985). The second (5′CGCATCAGGACGTTT3′) was chosen after the completion of the first sequence and was complementary to bases 506–491 downstream of promoter P1 (Figure 5a). This approach was taken to avoid additional subcloning. The sequencing direction, for both regions upstream to *rrnD* was from the 5′ end of the 16S gene toward the promoter regions and was determined three times for the 168 background and twice for the *trpE26* background as reported in Figure 5. After determining the sequence of segment A in pJR421, a third primer (5′AAGGCTGTTTCAATAATC3′) complementary to bases 306–324 upstream of *rrnD* from the *trpE26*-type strains was synthesized (Figure 5a). It was chosen to sequence the junction from the predicted segment A in a 168-type strain cloned in pAAM2 (Moir, Feavers and Guest 1984).

RESULTS

Restriction site polymorphisms 5′ to ribosomal operon *rrnD*: As mentioned in the Introduction, *Hin*dIII digests of two DNAs from *trpE26*-type strains (166 and GSY1269) when probed with DNA from a recombinant plasmid (p21C4) carrying 16S rDNA showed on Southern blots a new 3.3 kb band when compared to 168-type strains (Gottlieb, LaFauci and Rudner 1985). To further investigate this difference, we ran similar experiments with *Bcl*I, *Eco*RI and *Hin*dIII digests probed with different labeled cloned rDNA fragments. We have recently mapped all 10 rRNA gene sets of *B. subtilis* strain 168T and assigned them to 10 distinct *Bcl*I restriction fragments (LaFauci *et al.* 1986; Jarvis *et al.* 1988). Figure 2a shows that the 5.4-kb *Bcl*I fragment assigned to *rrnD* in strain 168T is replaced by a 7.1-kb fragment in strain 166. Rearrangements have therefore occurred in the *rrnD* area of the *trpE26*-type strains. Additional restriction site polymorphisms were noticed on Southern blots of *Eco*RI and *Hin*dIII digests of genomic DNA from strain 166 when probed exclusively with radioactively labeled 16S sequences. The 9.5-kb *Eco*RI fragment of strain 168T is replaced by a 2.3-kb fragment and the 2.6-kb *Hin*dIII fragment is replaced by a 3.1-kb fragment (Figure 2, b and c). Hybridizations of the same *Eco*RI and *Hin*dIII blots with a radioactively labeled 23S rDNA yielded patterns identical to those obtained for strain 168T (data not shown; Stewart, Wilson and Bott 1982; Widom *et al.* 1988). Heterogeneity in these two types of strains therefore resides on the 5′ side of *rrnD*.

The restriction maps of the *rrnD* operon in strains 168T and 166 are shown in Figure 3, a and c, respectively. Fragment sizes were deduced from the Southern hybridizations (Figure 2) and from the published sequence of *rrnB* (Green *et al.* 1985). As shown the size and location of the sensitive sites 5′ to *rrnD* in strain 166 are different from strain 168T suggesting that the region must contain new chromosomal sequences. As shown, part of the event which produced strain 166 occurred between the *Hin*dIII site and the 16S rRNA gene of 168 (Figure 3, a and c, and see below).

Following the above findings with strain 166 we similarly examined several other strains carrying either transferred or novel rearrangements of the *trpE26* system: GSY1269, GSY1835, GSY1127 (Table 1). Transferring the *trpE26* mutation (*i.e.*, a split *trpE* gene) from donor 166 DNA into a 168-type recipient most often led to the simultaneous induction of both rearrangements, translocation and inversion (Trowsdale and Anagnostopoulos 1975, 1976; Anagnostopoulos and Trowsdale 1976). One such strain GSY1269 (Table 1) was found to possess the same rearranged *Bcl*I, *Eco*RI and *Hin*dIII sites upstream to *rrnD* as the donor parent 166 (Figure 2).

The transfer of the split *trpE* gene (*i.e.*, *trpE26*) into 168-type strains by transformation with 166 DNA sometimes induces different rearrangements. In the case of strain GSY1835 (Table 1) a nontandem duplication of the *purB-tre* region (segment Ib, Figure 1) was created with the second copy of Ib inserted inside the *trpE* gene (Schneider and Anagnostopoulos 1981). The restriction patterns of this strain showed *Bcl*I, *Eco*RI and *Hin*dIII fragments characteristic of 168-type strains when probed with rDNA fragments

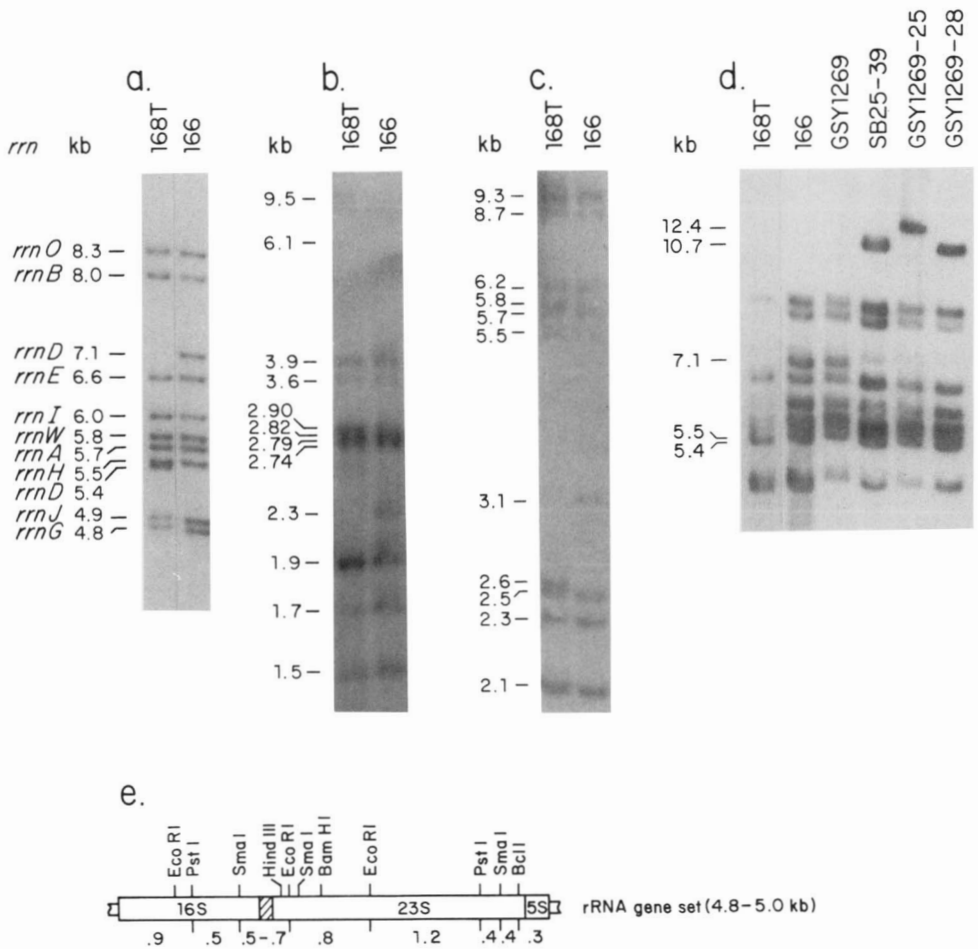

FIGURE 2.—Southern hybridization of total chromosomal DNAs from strains 168T and 166 of *B. subtilis*: (*a*) *Bcl*I digests probed with a labeled *Eco*RI-*Pst*I 23S rDNA fragment; the assignment of the *Bcl*I homologs of the individual *rrn* operons is according to Jarvis *et al.* (1988); (*b*) *Eco*RI digests probed with a labeled *Pst*I-*Sma*I 16S rDNA fragment; (*c*) *Hin*dIII digests probed with a labeled *Pst*I-*Sma*I 16S rDNA fragment; (*d*) *Bcl*I digests of integrant strains of 168 and *trpE26*-type strains probed with an *Pst*I-*Eco*RI 16S-2tRNAs -23S rDNA fragment. The faint band above 7.1 Kb contains tRNA sequences (LAFAUCI *et al.* 1986); (*e*) generalized restriction map of a *B. subtilis* rRNA gene set as proposed by STEWART, WILSON and BOTT (1982). The hatched area represents the abutment region between 16S and 23S rDNA with or without tRNA genes.

(Figure 2), but gave a pattern similar to the *trpE26*-type strains when probed with DNA upstream to *rrnD* (see below). This reveals that a region upstream to *rrnD* is found both on Ib-II and Ib-C junctions of strain GSY1835, the region belonging to the second copy of Ib (data presented below, Figure 6).

Novel rearrangements were also obtained using the *trpE26*-type strains as recipients instead of donors. Strain GSY1127 (Table 1) was obtained by transformation of strain GSY1269 (see above) with a 168-type donor DNA segment (*trpE*$^+$, *hisH2*). This led to the induction of both a nontandem duplication of the *hom-trpE* region (segment B, Figure 1) and the loss of the large inversion in the *trpE26*-type recipient (SCHNEIDER, GAISNE and ANAGNOSTOPOULOS 1982). This strain (GSY1127) gave the normal *rrn* Southern patterns as seen for 168T (Figure 2; data not shown). One may conclude that reversing the inversion by recombination with heterologous chromosomal fragments also restores the original sequences 5′ to *rrnD*.

ANAGNOSTOPOULOS and co-workers used recipients which were lacking *rrnG*; these strains have 9 instead of 10 *rrn* operons (data not shown). This deletion and a similar one involving *rrnW* has been well characterized by us (WIDOM *et al.* 1988). The constructed strains GSY1269 and GSY1835 (Table 1) still possessed only 9 operons and lacked the 4.8-kb *Bcl*I homolog although the donor 166 contained 10 (Figure 2). Therefore, as expected the rearrangements introduced during the transfer of the *trpE26* mutation or in the reciprocal cross using 168-type DNA do not involve the entire donor chromosome but only occur locally at specific junction regions during the transformation process.

Integrative mapping of the rearranged junction adjacent to *rrnD* in the *trpE26*-type strains: The results of the hybridization experiments reported above showed clearly that the area upstream from the *rrnD* operon was involved in the rearrangements of the *trpE26*-type strains. We therefore sought to map the chromosomal location of this operon in these strains. This was achieved by the integrative mapping method introduced by HALDENWANG *et al.* 1980 and adapted for mapping *rrn* operons by LAFAUCI *et al.* 1986. It is based on the integration into the *B. subtilis* chromosome, by a Campbell-like mechanism, of a plasmid carrying an antibiotic resistance marker and a short rDNA fragment that guides integration into any of the 10 *rrn* operons. This confers a powerful selective phenotype to these areas by allowing mapping of the operon when resistant clones are used as donors in subsequent genetic crosses.

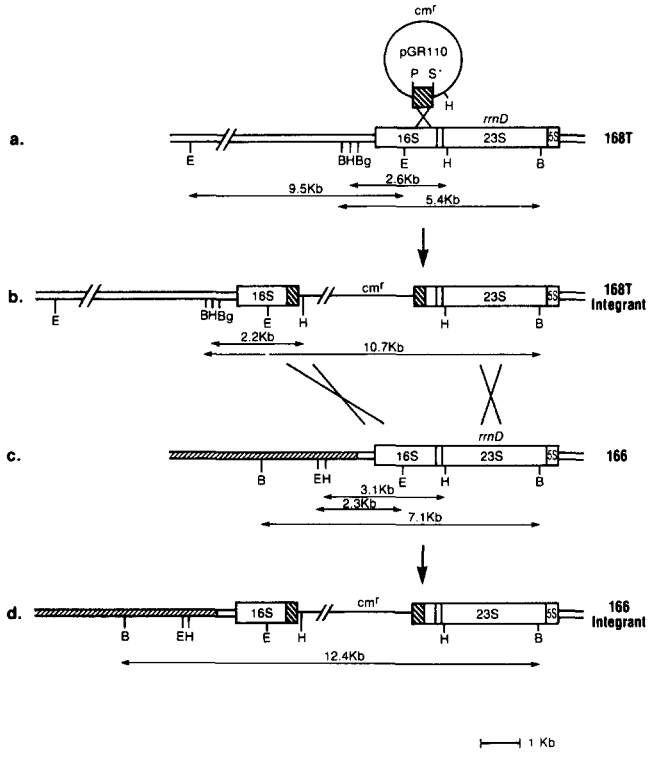

FIGURE 3.—Fragment sizes shown are those identified by restriction analysis in Figure 2, a–d. The hatched areas represent new regions of DNA due to rearrangements or to duplications by plasmid integration. The letters B, Bg, E, and H indicate *Bcl*I, *Bgl*II, *Eco*RI, and *Hin*dIII restriction sites respectively. For additional information on the generalized restriction map of a *B. subtilis* rRNA gene set as proposed by STEWART, WILSON and BOTT (1982) see Figure 2 and JARVIS *et al.* (1988).

As previously reported (LAFAUCI *et al.* 1986; JARVIS *et al.* 1988), integration of a plasmid containing *rrn* sequences and a Cm^r determinant into *rrnD* of 168T results in the loss of the 5.4-kb *Bcl*I fragment with the appearance of a higher molecular weight band corresponding to the size of the plasmid plus the missing *Bcl*I *rrn* fragment (Figures 2d and 3, a and b). Instead of relying on the low chance of plasmid integration into *rrnD* (5%, see Table 5 in JARVIS *et al.* 1988), a directed approach to achieve an insertion into the aberrant 7.1-kb homolog from *trpE26*-type strains was used. This was accomplished by transforming *trpE26* recipients (*i.e.*, strains 166 or GSY1269) with donor DNA containing an integrated Cm^r element in *rrnD* from a 168 background (*i.e.*, 168T-18, SB25-39 or BD170-10; Table 1). It was anticipated that the ribosomal operon itself and the 3′ unaltered region would provide the necessary homology to insert the Cm^r determinant by a double cross over event (Figure 3, b–d). Cm^r transformants arose with high frequency. In order to map these integration events we prepared PBS1 transducing lysates from 50 of the Cm^r clones and used them as donors to investigate linkage of the Cm^r determinant to the appropriate genetic markers. Table 2 summarizes the results with relevant recipients: RR23 (*cysB3*; *trpE26*-type); kit 1 (*cysA14*; 168-type) and kit 3 (*glyB133*; 168-type). The latter two strains are the mapping recipients constructed by DEDONDER *et al.* (1977).

TABLE 2

Integrant classes obtained by transfer of 168-type *rrnD* into *trpE26*-type strains

Donors for transduction obtained by transformations[a]			Recipients for transductions[b,c]		
Recipient *trpE26*-type	Donor DNA 168-type (plasmid)	No. tested	RR23 *cysB3*	Kit 3 *glyB133*	Kit 1 *cysA14*
166	168T-18 (pGR102)	7	5	2	0
166	SB25-39 (pGR110)	10	10	0	0
166	BD170-10 (pGR111)	3	3	0	0
166	SB25-43 (pWR112)	4	0	2	2
GSY1269	SB25-39 (pGR110)	12	11	1	0
GSY1269	BD170-10 (pGR111)	8	5	2	1
GSY1269	SB25-43 (pWR112)	6	0	2	4
	Percent	100	68	18	4

[a] PBS-1 donor lysates were prepared from *trpE26*-type clones obtained by transformation with DNA from 168-type strains which carries an integrated plasmid into *rrnD*. The description of the integrated plasmids was discussed in MATERIALS AND METHODS.

[b] Recipient strains were either of *trpE26*-type (RR23) or 168-type (kit 1 and kit 3). Cm^r transductants were isolated and the linkage for the auxotrophic marker was determined. Results are presented as the number of donor lysates which showed linkage of Cm^r to the indicated marker.

[c] For genomic locations and linkage values of *rrnD* to *cysB3*, *glyB133* and *cysA14*, see Figure 4 and JARVIS *et al.* (1988).

The *trpE26*-type integrants fell into these three classes. The majority class, 68% of the clones tested, only transduced the Cm^r marker to *trpE26*-type recipients (RR23) at high frequencies of about 1×10^4/ml and were unable to transduce the Cm^r marker to 168-type strains (kit 1 or kit 3). The Cm^r marker of these integrants showed a tight linkage to *cysB* and a weak one to *hisA*. These genes are located at positions 300° and 305°, respectively, on the 168 genetic map and are part of the inverted chromosome segment A in the *trpE26*-type strains (Figure 1). Figure 4 shows the PBS1 transduction maps of the *rrnD* areas in the two types of strains. A representative clone of this class, GSY1269-25, showed the loss of the 7.1-kb *Bcl*I fragment with the concomitant appearance of a higher molecular weight band of 12.4 kb, significantly larger than that of the donor SB25-39 DNA of 10.7 kb (Figure 2d). These results demonstrate that the rearranged junction 5′ to *rrnD* in *trpE26*-type strains mapped in the same location as the A-II junction reported by ANAGNOSTOPOULOS (1977; Figure 1).

The second unexpected class, 18% of the clones tested, only transduced the Cm^r marker to 168-type recipients and displayed linkage of this marker to the *glyB* gene of strain kit 3 a 168-type strain (Table 2 and Figure 4). DNA from an integrant of this class, GSY1269-28, also revealed the loss of a 7.1-kb fragment but the new higher molecular weight band was the same size as its SB25-39 donor, 10.7 kb (Figure 2d) characteristic of an area containing the *rrnD* operon in a 168-type strain (Figure 3). It appears there-

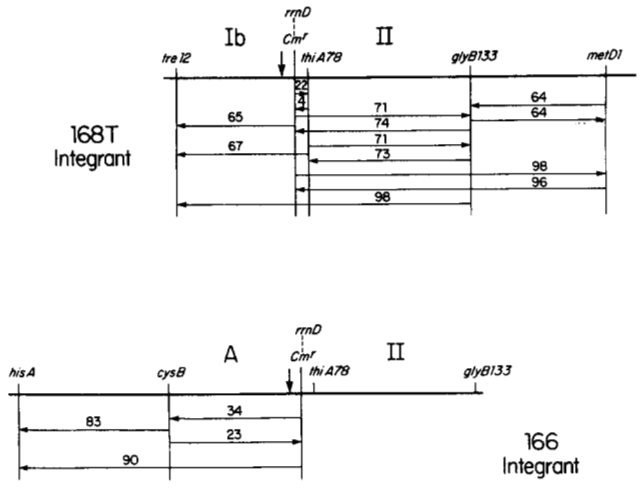

FIGURE 4.—Genetic maps of the *rrnD* operon region in 168- and *trpE26*-type strains. The maps were constructed from results of PBS1 transduction crosses. The arrows point to the selected marker and the numbers are 100-cotransduction frequency. The vertical arrow represents the postulated location of the junction where a disruption occurred. The 168-type map is taken from LAFAUCI *et al.* (1986).

fore as if the clones of this class possess the Ib-II junction of 168-type strains; *i.e.*, as if the inversion of the *trpE26*-type recipient strain has returned to its original orientation. In this respect GSY1269-28 resembles strain GSY1127 (SCHNEIDER, GAISNE and ANAGNOSTOPOULOS 1982). However the two strains differ in other respects; GSY1127 is Trp^+ and possesses a non-tandem duplication of segment B (*hom-trpE*), while the GSY1269-28 is Trp^- (presumably still possessing the *trpE26* mutation) and it is not known whether it carries duplications although it is now phenotypically Ilv^+ (Table 1). A more thorough genetic analysis of this clone is likely to bring useful information. It is possible that heterozygous donor DNA carrying the junction upstream of *rrnD* has the potential to induce the reversal of the inversion. The results with the first two classes illustrates that the lack of homology between the two types of strains in the *rrnD* area hinder the transfer of the Cm^r determinant during transduction but not during transformation.

The third class, 14% of the total clones examined, transduced the Cm^r marker to both *trpE26*- and 168-type strains at equal frequencies, but the transferred plasmid had integrated into other *rrn* operons; mainly those linked to *cysA* near the origin where 7 such operons are situated (Figure 1 and JARVIS *et al.* 1988). The majority of this class arose from plasmid pWR112 containing additional heterologous DNA from the *lacZ* gene of *E. coli* (WIDOM 1988).

Cloning and sequencing the junction 5′ to *rrnD*: The above data have clearly shown that one of the recombination events which led to the rearrangements of the *trpE26* strains took place close to *rrnD* in the 5′ upstream region of this operon. It became therefore important to clone and determine the nucleotide sequence of the region in both types of strains. The cloning was accomplished by rescue of the integrated plasmid from integrant strains SB25-39 (168-type) and GSY1269-25 (*trpE26*-type). The resulting rescued plasmids were designated pWR305 and pJR421 respectively (Table 1). Restriction analyses and Southern hybridizations (data not shown) indicated that each rescued DNA spanned sequences from the common *Bcl*I site in the 23S rRNA gene to the unique *Bcl*I sites upstream of each *rrnD* operon (see Figure 3, b and d). The sequences of both unique regions were determined by starting from the 5′ end of the 16S gene as described in MATERIALS AND METHODS.

A segment of 808 nucleotides beginning from the *Hin*dIII site upstream of *rrnD* in the common 168-type background and ending inside the 16S rRNA gene is shown in Figure 5a. Comparisons with reported sequences show that the regulatory elements of this region were similar to five other upstream regions of *rrn* operons in *B. subtilis*: *rrnO*, *rrnA*, *rrnJ*, *rrnH* and *rrnB* (GREEN *et al.* 1985; OGASAWARA, MORIYA and YOSHIKAWA 1983; WAWROUSEK and HANSEN 1983; WIDOM 1988). An additional 65-bp sequence was found attached to the leader between P2 and the 16S of *rrnD* (Figure 5a). This leader insert also occurs in the same position upstream of the 16S in *rrnO* and *rrnB* increasing the structural heterogeneity of ribosomal RNA operons in *B. subtilis* (GREEN *et al.* 1985; OGASAWARA, MORIYA and YOSHIKAWA 1983). Finally, the 5′ region beginning with the *Hin*dIII site seems to be part of an ORF as shown by the amino acid sequence although the initiation codon is not part of this portion of the sequence. The coding region terminates between the −35 and −10 region of P1 (Figure 5a). To date, a database search revealed no homology of this sequence with any known DNA or protein sequence.

When the sequence upstream of *rrnD* from a 168-type strain was compared to a *trpE26*-type strain, perfect homology was found beginning with nucleotide 377 and continuing onto the 16S rRNA gene (Figure 5a). Upstream to this site at least 146 bp were sequenced from the *trpE26*-type strain and the region was found to be totally divergent with respect to both nucleotides and amino acids from the 168-type strain (Figure 5a). A coding region in the *trpE26*-type strains was found which continues across the junction and terminates 11 bases upstream of P1 (Figure 5a). As in the first case, a database search revealed no homology of this sequence with any known DNA or protein sequence. These sequences upstream of *rrnD* were further compared to plasmid pAAM2 containing a 5 kb *Eco*RI fragment of *citG-gerA* DNA and an additional 1.5-kb *Eco*RI fragment containing the downstream end of the *citG* gene. The plasmid has a section with similar restriction sites as in the region upstream of *rrnD* in the *trpE26*-type strains. Specifically the

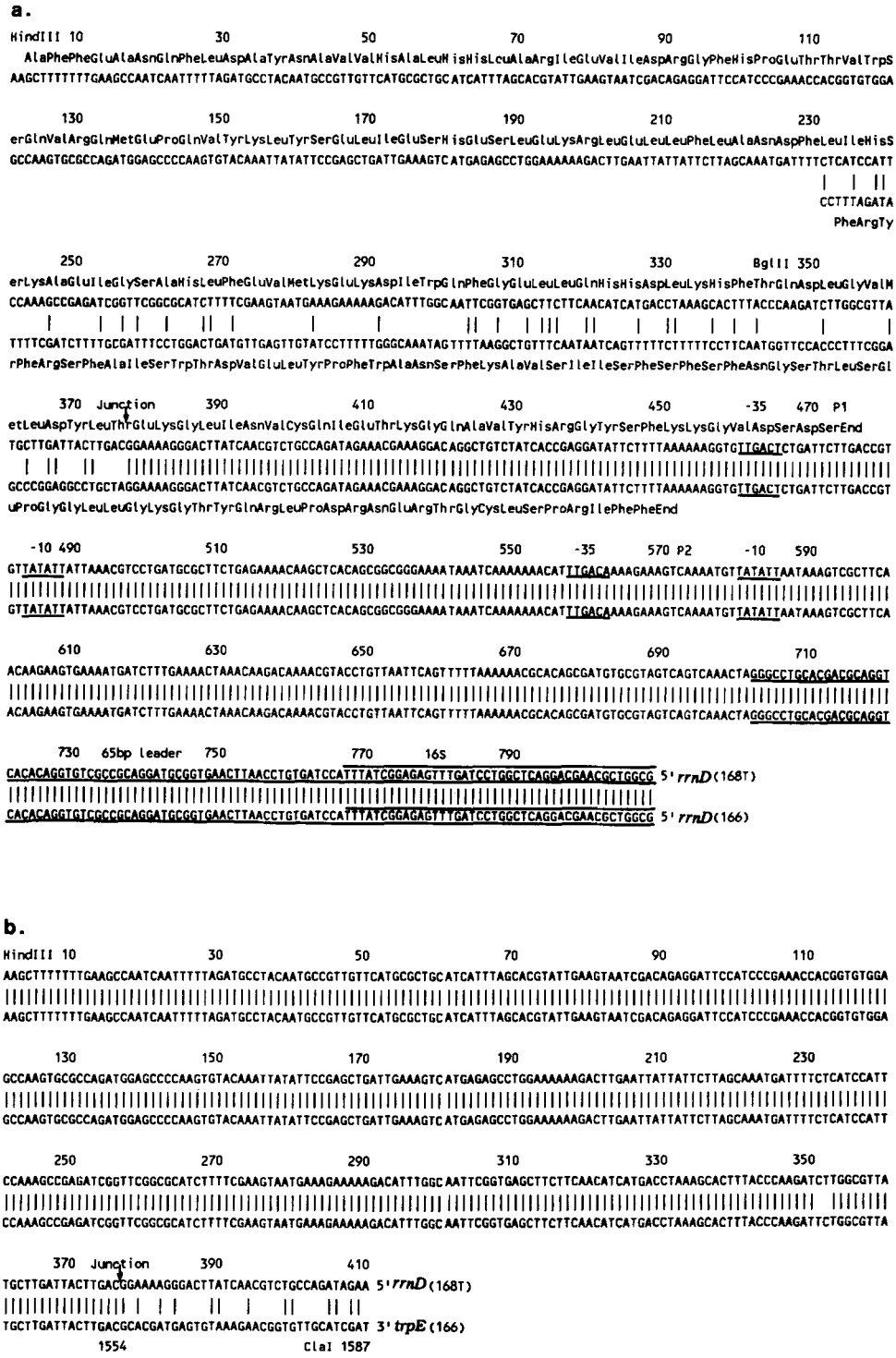

FIGURE 5.—(a) DNA sequence and opeon reading frames upstream to *rrnD* in *a.* 168 and *trpE26*-type strains of *B. subtilis*. The 808 nucleotides beginning from the 16S sequence of *rrnD* are aligned with 578 bases upstream to *rrnD* from 166. Two promoters can be found, each with −35 and −10 consensus boxes separated by 17 bp. A 65-bp insert is found between P2 and 16S for *rrnD* from both strains. The two sequences diverge 5′ to nucleotide 377. The location of the junction involved in the rearrangements is indicated. The locations of the *Hin*dIII and *Bgl*II sites are shown. The sequence below represents the region downstream of the *citG* gene from 168 cloned in pAAM2 using a primer complementary to bases 306–324 upstream of *rrnD* in the *trpE26* strains. The arrow indicates the break point at the A-B junction of 168.

Break (nucleotide 377)

Downstream *citG*	1	TTTTTCCTTC	AATGGTTCCA	CCCTTTCGGA	GCCCGGAGGC	CTGCTAGGA
	51	TATGACCATAT	AGTCACACCG	TCTGTTATAA	AATTAACCTT	ATTGTAACT
	101	TGTTTTTTTAT	CATGTATTAT	TTATATTATA	AATTGAGATG	ACCGAATGG
	151	TCAAAGCTCAA	GCGAAAGGGT	GAATCTAGAA	TGAGAGA	

(b) Comparison of the DNA sequences upstream to *rrnD* in a 168-type strain and upstream to the translocated *trp* operon in a *trpE26*-type strain. Both begin at the *Hin*dIII site and align perfectly up to nucleotide 378 upstream to *rrnD* or 1587 in the *trp* operon of strain 166 (HENNER, BAND and SHIMOTSU 1984). The junction involved in the rearrangements is indicated.

relative order and distance of three restriction sites *Bcl*I (B), *Eco*RI (E) and *Hin*dIII is identical (Figure 3c; MOIR, FEAVERS and GUEST 1984; MILES and GUEST 1985). According to SAMMONS and ANAGNOSTOPOULOS (1982), a 1.4-kb *Eco*RI fragment from a similar clone carries the junction between segments A and B from 168-type strains which is split into two parts in *trpE26*-type strains (Figure 1). They showed by Southern hybridizations of *trpE26*-type DNA probed with the 1.4-kb *Eco*RI fragment two bands, one corresponding to a larger fragment and one smaller. Our 2.3-kb *Eco*RI fragment seen in Southern blots of *trpE26*-type DNA (Figures 2b and 3c) may correspond to the larger band described by SAMMONS and ANAGNOSTOPOULOS (1982). Based on our findings and the restriction analysis of the *citG* fragment (MILES and GUEST 1985), we predicted that sequences downstream of *citG* gene are fused to the 5′ region upstream to *rrnD* in the *trpE26*-type strains. When a synthetic primer complementary to nucleotides 306-324 was used to sequence the 1.5-kb *Eco*RI fragment of pAAM2, a region perfectly homologous to the region upstream of the junction from *rrnD* at nucleotide 377 was found (Figure 5a and its legend). One may conclude that as predicted in the *trpE26*-type strains segment A containing the *citG* gene is actually fused to segment II, which contains the 5′ region upstream to *rrnD* (Figure 1).

According to the above data and the maps of Figure 1, the sequence 5′ to bp 377 upstream from *rrnD* of the 168-type strains should be located at the Ib-C junction in the *trpE26*-type strains linked to the split terminal part of the *trpE* gene. As stated in the Introduction the nucleotide sequence of this junction has already been determined (by L. BAND and D. J. HENNER) and was communicated to us by D. HENNER. They sequenced a *Hin*dIII-*Cla*I 410 bp segment from a 850 bp cloned DNA of strain 166 inserted in plasmid p*trp*1A65. This sequence is compared to the 5′ region upstream to *rrnD* from 168-type strains in Figure 5b. A perfect homology, except for two bases, exists between these two sequences in the region 5′ to nucleotide 377 upstream to *rrnD*. The exception concerns bases 351 and 352, we found C-T whereas Band and Henner found the bases in reverse order, T-C. The last 35 bases of p*trp*1A65 have 100% similarity with the *trpE* gene nucleotides 1554 to 1587 in the sequences of the *trp* operon (HENNER, BAND and SHIMOTSU 1984).

These facts lead to the conclusion that the sequence 5′ to nucleotide 377, upstream to *rrnD*, is part of the inverted A-O-I chromosome segment (Figure 1) and is fused to the split *trpE* gene carried by the translocated segment C. Concomitantly a sequence at the end point of segment A is fused to nucleotide 377 thus sealing the inversion in the *trpE26* strains. The region upstream to *rrnD* is therefore one of the junctions involved in the chromosomal arrangements of these strains.

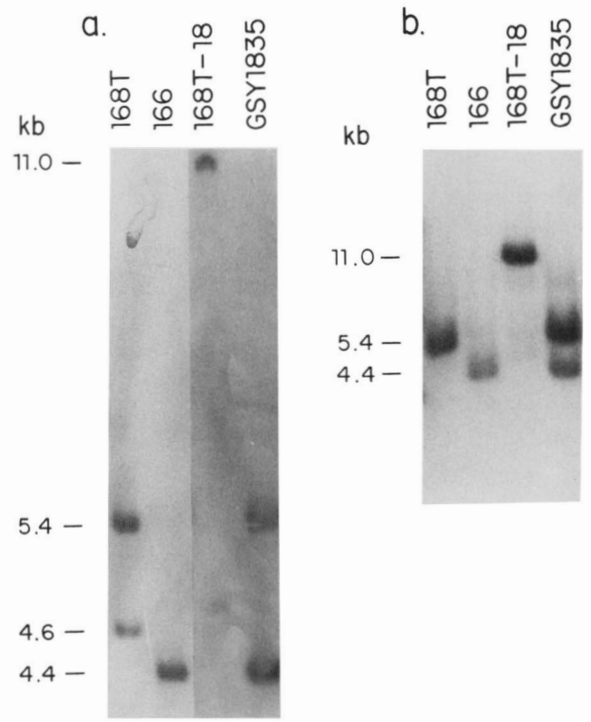

FIGURE 6.—Southern hybridization of *Bcl*I digest with a fragment from segment Ib-C (*trpE26*) and from the Ib (168) junction respectively. The *Bcl*I digests were probed with labeled (a) p*trp*1A65 containing the junction of 5′ *rrnD-trpE*; and (b) a 353-bp *Hin*dIII-*Bgl*II fragment containing sequences 5′ to the junction upstream to *rrnD* (see Figures 3a and 5a). For strain information, see Table 1.

In order to verify further the chromosomal location where the fusion events actually took place and to determine if there are other chromosomal regions that share homology with these junction sequences, Southern hybridizations were performed. *Bcl*I digests of 168-type and *trpE26*-type DNAs were probed with the following: (a) plasmid p*trp*1A65 containing the junction with the 5′ end point upstream to *rrnD* fused to the 3′ end of *trpE* from strain 166 (Figure 5b); and (b) a control *Hin*dIII-*Bgl*II fragment containing sequences on the 5′ side of the junction upstream to *rrnD* but not to the junction itself (Figures 3a and 5a). With the p*trp*1A65 probe strain 168T yielded the expected 5.4-kb band containing *rrnD* and a 4.6-kb homolog of the *trpE* gene (Figure 6a). In the hybridization pattern of strain 166 these bands were absent; instead, a single band of 4.4 kb representing the fusion region of the Ib-C segment appeared (Figures 1 and 6a). The p*trp*1A65 probe moreover shared no homology with the 7.1-kb *Bcl*I homolog containing *rrnD* in strain 166. No additional bands were observed indicating that even at other genomic locations there are no regions that share extensive intrachromosomal homology involved in the rearrangements with this junction. The second (0.4 kb *Hin*dIII-*Bgl*II) probe hybridized only to the 5.4-kb *Bcl*I homolog containing

rrnD in strain 168T and to the fused 4.4-kb 5′ *rrnD-trpE* fragment in strain 166 (Figure 6b). Both probes hybridized to the high molecular weight fragments (11.0 kb) of *rrnD* integrants like strain 168T-18 (Table 1, Figure 6).

Southern hybridizations of DNA from strain GSY1835 (*trpE30*) which carries the nontandem duplication of segment Ib (Figure 1; SCHNEIDER and ANAGNOSTOPOULOS 1981) showed both the normal *rrnD* homolog (5.4 kb) and the 5′ *rrnD-trpE* fusion homolog (4.4 kb) on blots irrespective of the labeled probe used. This finding demonstrates that in GSY1835 one of the copies of the duplicated segment Ib which is linked to segment II carries the intact upstream region of *rrnD* while the other copy ends at the heterologous junction 5′ *rrnD-trpE* fusion (Ib-C) as in the *trpE26*-type strains. These results are consistent with the conclusions made by SCHNEIDER and ANAGNOSTOPOULOS (1981) that the junction involved in the nontandem duplication of the *trpE30* strains are the same as those responsible for the rearrangements of the *trpE26* strains.

DISCUSSION

In this paper we presented physical and genetic studies on the upstream region of ribosomal operon *rrnD* in *B. subtilis* 168 and *trpE26*-type strains. Restriction site polymorphism involving the relative position of the sensitive sites for the enzymes *Hin*dIII, *Eco*RI, and *Bcl*I were observed. The restriction pattern differences were due to a disruption of an area upstream to the *rrnD* operon and not to its coding region. In *trpE26*-type strains that region is linked to new chromosomal sequences as demonstrated by genetic crosses and nucleotide sequence analysis. By employing integrative plasmids carrying a Cm^r determinant we were able to isolate, map and sequence the 5′ region to *rrnD* in both types of strains (168 and *trpE26*). The genetic data specifically showed linkage of *rrnD* to *cysB* and *hisA*, both located on segment A, in the *trpE26*-type strains. Normally in 168 that operon is linked to *tre* and *purB* both located on segment Ib (Fig. 1). We compared these sequences with the downstream region of the *citG* gene from strain 168 and with the sequence of *trpE26* mutation communicated to us by D. HENNER. These analyses allowed us to identify the exact break points of at least three junctions involved in the rearrangements of the strains carrying the *trpE26* mutation.

The data presented in this paper have first confirmed the genetic maps of the strains carrying rearrangements, constructed by ANAGNOSTOPOULOS and co-workers, by demonstrating physical linkage in the following junction sites: (1) A-II in the *trpE26*-type strains (*rrnD* fused to a sequence which is the endpoint segment A). (2) Ib-C in the *trpE26*-type strains (the upstream region to *rrnD* fused to the split terminal part of *trpE*); (3) Ib-II in GSY1127 (reversal of the inversion of the recipient parent); (4) both Ib-II and Ib-C junctions in GSY1835 containing the *trpE30* mutation (nontandem duplication of segment Ib). The sequences of the junctions known to date are presented in Figure 7. The two main problems however remain unsolved; *i.e.*, the mechanism of induction of the *trpE26* rearrangements in the original mutant, strain 166, and that of their simultaneous transfer or induction of novel rearrangements between genetic crosses into 168-type and *trpE26*-type strains. Strain 166 is presumably a X-ray mutant (see Introduction) and it is well known that ionizing radiation induces chromosomal rearrangements. The major lesions produced by X or γ-rays are DNA double strand breaks (DSB) which in bacteria are repaired either by a fast process with DNA ligase or by a slower process using the *rec* pathway (WEIBEZAHN and COQUERELLA 1987). Whether or not the production of double strand fragments by X-ray is random or sequence-specific is unresolved. In the case of strain 166, we propose the X-ray exposure of the spores created DSBs at four locations indicated by arrows on the 168-type map shown in Figure 7. The chromosome is fragmented into four linear segments which are then joined end-to-end at the locations indicated by the arrows on the 166 map in Figure 7.

In only one of the breakpoints (junction B-C of 168; inside the *trpE* gene) genetic information has been affected. All the other novel junctions in strain 166 were apparently formed in silent or redundant areas of the chromosome. Three regions where the original breaks occurred have been sequenced in 168; the Ib-II, A-B and the B-C junctions (Figures 1 and 7). There is a striking but limited homology only at the 5′ ends from two of these junctions; 11 bp out of 15–16 bp were matched between the Ib-II and B-C junctions (see Figure 7). In both regions this homology was found five bases away from the split which occurred between C-G nucleotide pairs (see Figure 7). This limited homology may be fortuitous or indicate that X-ray-induced DSBs occur at specific sequences. Two of the four junctions of strain 166 have been sequenced; the A-II and Ib-C junctions which do not share homology. Although the sample is limited, the available data suggest that extensive homology was not required to facilitate the end-to-end rejoining of the double strand fragments. During the rejoining process, none of the sequences surrounding the two junctions in strain 166 were altered except for the possibility of 2 bp located 25 nucleotides upstream of the Ib-C junction (Figure 5b). Alternatively the break points could have occurred 1 bp 3′ to each of the arrows drawn above the known sequences shown in Figure 7. The outcome of either break points would be the same and thus the conclusion regarding the rejoining process remains unchanged. With the avail-

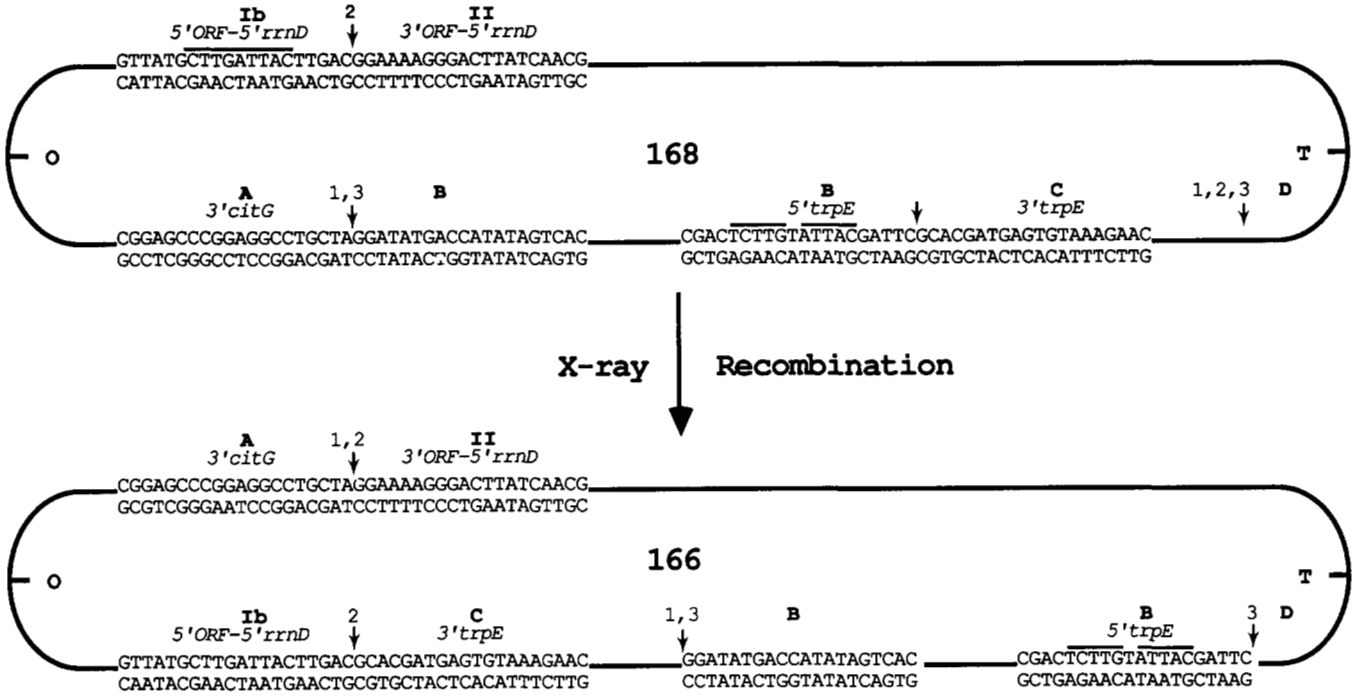

FIGURE 7.—Nucleotide sequence and chromosomal location of the junctions involved in rearrangements so far identified in the 168- and *trpE26*-type strains. The meaning of capitals, Roman numerals and numbers was explained in the legend to Figure 1. 3′-ORF-5′ *rrnD* designates the putative open reading frame 5′ to *rrnD*. The sequences of segment B in the *trpE26* chromosome are deduced from the 168-type strain and have not yet been verified. The underlined sequences indicate regions of small homology.

able information, we predict that the sequence between *dal* and *purB* (Figure 1, box 3) was also altered by X-ray radiation.

Several events that can occur in the *trpE26* system require explanation: (1) the simultaneous transfer of both rearrangements (translocation and inversion) into 168 recipients; (2) the formation of non-tandem duplications of segment Ib (*purB-tre*) mediated by *trpE26* donors in 168-type recipients; thus creating the *trpE30*-type strains (SCHNEIDER and ANAGNOSTOPOULOS 1981); (3) the induction of long unstable tandem duplications by 168 donors in *trpE26* recipients (AUDIT and ANAGNOSTOPOULOS 1972, 1973) and reciprocally by *trpE26* donors in 168 recipients (TROWSDALE and ANAGNOSTOPOULOS 1976); (4) the stabilizations of these unstable structures by loss of part of one copy leaving the other part as a nontandem duplication (SCHNEIDER, GAISNE and ANAGNOSTOPOULOS 1982); (5) the formation of nontandem duplications of segment C (*trpE-ilvA*) in *trpE30*-type recipients (SCHNEIDER and ANAGNOSTOPOULOS 1983). The induction of the tandem duplications (item 3 above) can easily be interpreted by the interaction of the donor DNA with two sister recipients chromatids (unequal recombination; TROWSDALE and ANAGNOSTOPOULOS 1975, 1976; ANAGNOSTOPOULOS and TROWSDALE 1976).

All other events were explained by ANAGNOSTOPOULOS (1977, 1990) by speculative models of recombination between specific junction sequences of intrachromosomal homology. Three sets of such sequences labeled 1, 2 and 3 were proposed, their locations were at the end points of the rearrangements (Figure 1). One can speculate that the action of X-rays is preferential and occurs at the same specific junction sequences of intrachromosomal homology.

To date, sequence data (Figure 7) does not reveal the presence of long specific sequences of intrachromosomal homology across junctions that could have been involved in the formation of the initial rearrangements by X-ray or in the formation of novel rearrangements during genetic crosses. For example, the proposed sequence No. 2, should have been involved in the transfer of the *trpE26* inversion into a 168 recipient to create strain GSY1269 (Table 1; ANAGNOSTOPOULOS and TROWSDALE 1975) and in the restoration of the inversion during the formation of stable nontandem duplications of segment B (strain GSY1127; SCHNEIDER, GAISNE and ANAGNOSTOPOULOS 1982). The model predicts that in strain 166, the same sequence No. 2 needs to be located on the opposite sides of the chromosome between segments A and II and between Ib and C. As shown in Figure 7, the chromosome of strain 166 does not contain such sequence homology across these junctions. Moreover, sequences across the 168 junctions (Ib-II and A-B) did not show homology "across" those of 166 (A-II and Ib-C) as predicted by the hypothesis (Figure 7). One may have to conclude that these rearrangements and transfer events must occur by novel mechanisms

that do not require extensive sequences of "intrachromosomal" homology. Such novel mechanisms may simply require only short homologous sequences near the breakpoints or involve pairing of sequences flanking a novel point in the donor with their homologous sequences located at different regions of two chromosomes of the recipient, like in the case of the induction of the tandem duplications (ANAGNOSTOPOULOS and TROWSDALE 1976).

The *trpE26* system presents certain similarities with that of the amplification of the *amyE-tmrB* region of *B. subtilis* studied by YAMAZAKI and co-workers (MORI *et al.* 1986; FURUSATO *et al.* 1986; HASHIGUCHI *et al.* 1986). No regions of extensive homology were found at the endpoints of the repeating unit. However, both parental and amplified strains possess very short (7 bp) direct repeats (not the same in both strains) flanking the break and joining points. The seven base repeated sequence of the parental strain is composed entirely of purines on the same strand. It is part of a 13-base polypurine sequence on the left side of the break point, present in both strains. So far there is no evidence that either the repeats or the polypurine sequence are directly involved in the induction of the duplication and the subsequent amplification. The thirteen base polypurine sequence (-AAAGAGGGAAGGA-) shows a seven base homology with the polypurine sequence (-GGAAAAGGA-) we found on the 3′ side of the Ib-II junction in strain 168T. In the enteric bacteria, repetitive sequences also called REP (repetitive extragenic palindromic) seem to be involved in the formation of chromosomal rearrangements such as duplications. In *S. Typhimurium* it was shown that the REP can recombine with each other even within a 7 bp homology and lead to duplications at considerable distances (SHYAMALA, SCHNEIDER and AMES 1990). These REP sequences differ considerably from the polypurine sequences described here and by MORI *et al.* 1986. One can only speculate that in the genus *Bacillus* the short polypurine sequences may belong to a family of dispersed repetitive DNA with a similar role at the join-point in the recombination event (SHYAMALA, SCHNEIDER and AMES 1990).

To interpret the *amyE-tmrB* amplification in *B. subtilis* the authors advanced the following hypothesis (HASHIGUCHI *et al.* 1986). The primary event would be an unequal illegitimate recombination, due to mutagenic action, between sister chromatides, taking place at the right hand side of the *trmB* gene and the left hand end upstream of *amyE*. This event created the novel junction and a tandem duplication. Subsequent amplification (as well as the transfer of the rearrangement by transformation) can then easily be explained as the result of recombination in the long homologous regions flanking the novel joint like in the case of the tandemly duplications in the *trpE26* system described above.

Unequal illegitimate recombination could also be put forward for the formation of strain 166, *i.e.*, the initial induction of the *trpE26* rearrangements. However in this case four novel joints were created and at least six chromosome breaks should have occurred in the two chromatides. Besides the primary events did not induce any duplications. At present no satisfactory molecular model can be proposed that fits all the data available. As stated above the few junctions so far sequenced did not reveal any extensive homologous regions at the ends of the segments. Whether the short repeats developed play a role either directly or by triggering (or stimulating) recombination at nearby regions cannot be assessed. An overall interpretation and the proposal of new molecular models must await the determination of the nucleotide sequence of the remaining junctions.

We dedicate this paper to C. ANAGNOSTOPOULOS, who discovered and elucidated the mechanism of chromosomal rearrangements in *Bacillus subtilis*. He was truly a pioneer and an inspirational force in the area. We thank him for strains, fruitful discussions, his critical review of this manuscript and encouragement throughout this work. We thank D. J. HENNER for providing us with the plasmid p*trp*1A65 and his unpublished sequence and A. MOIR for giving us pAAM2. We thank G. WIPF from the Fred Hutchinson Cancer Research Center for sequencing a portion of pAAM2. We thank B. PRICE and P. RAPPA for assisting in some of these studies. This research was supported by City University of New York Faculty Research Awards Nos. 665126 and 668151, by Minority Research Centers in Minority Institutions National Institutes of Health grant RR03037.

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