***Bacillus subtilis* Strains Carrying Two Non-tandem Duplications of the *trpE-ilvA* and the *purB-tre* Regions of the Chromosome**

By ANNE-MARIE SCHNEIDER AND C. ANAGNOSTOPOULOS*

Centre de Génétique Moléculaire, Centre National de la Recherche Scientifique,
91190 Gif-sur-Yvette, France

(Received 13 July 1982)

Bacillus subtilis strains possessing the *trpE30* marker (splitting of the *trpE* locus and a non-tandem duplication of chromosome segment Ib: *purB-tre*) when transformed or transduced to tryptophan independence mainly give rise to haploid cells with the genetic structure of strain 168. However, among the Trp⁺ transformants or transductants about 10% are merodiploid carrying a non-tandem duplication of segment C (*trpE-ilvA*) while maintaining that of segment Ib. Linkage and segregation studies made it possible to determine their genetic structure, which can be represented by three different maps. In map *a* the copies of Ib are inverted repeats and one of them is flanked by two direct repeats of segment C; in map *b* two Ib–C segments are inverted repeats and in map *c* the copies of C are inverted repeats with one of them flanked by direct repeats of Ib. It is proposed that transition from map *a* to map *b* and then to map *c*, and vice versa, may occur by recombination between inverted repeats of either Ib or C. The merodiploids are unstable, recombination between direct repeats leading to haploid cells of 168-type structure. The models proposed for merodiploid formation call for fusion of two recipient chromosomes mediated by the donor segment and recombination between copies of a DNA sequence of the two chromosomes located in different regions. In the case of PBS-1 mediated transduction the greater length of the donor DNA segment makes it possible to obtain the merodiploids with a single recipient chromosome and this needs only a slight modification of the models. No *trpE30*⁺ merodiploids are found in transformation when the recipient carries a deletion of the SP β prophage, or in transduction when both donor and recipient possess this deletion. These results indicate that the homologous sequences involved may be part of the SP β prophage or that a sequence of bacterial DNA has a good homology with it.

INTRODUCTION

Studies of *trpE26* strains of *Bacillus subtilis* have provided evidence for the induction of extensive chromosomal rearrangements in this organism (Audit & Anagnostopoulos, 1972, 1973, 1975; Trowsdale & Anagnostopoulos, 1975, 1976; Anagnostopoulos & Trowsdale, 1976; Anagnostopoulos, 1977; Schneider & Anagnostopoulos, 1981; Schneider *et al.*, 1982). The tryptophan requirement of the *trpE26* strains is due to the splitting of the *trpE* locus, which is the result of a translocation of the *trpE(b)-ilvA* chromosome segment. These strains also possess an inversion of the upper part of the chromosome adjacent to the translocation. Abridged maps of the 'normal' 168-type and the *trpE26* strains are shown in Fig. 1. One of the problems encountered in these studies was the mechanism of transmission of the rearrangements of the *trpE26* strains into 168-type recipients by transformation. Simultaneous transfer of both rearrangements in the *trpE26* strains had been previously observed. However, this is not the only way that the defect of the *trpE* locus can be transferred genetically. In a previous paper we reported the isolation, the determination of the genetic structure and the properties of a strain in which the introduction of the separated parts of the *trpE* locus resulted in a novel rearrangement: a non-tandem duplication of the Ib segment (*purB-tre*) (Schneider & Anagnostopoulos, 1981). The genetic map of this strain and its derivatives designated as *trpE30*

Fig. 1. Diagrams illustrating the differences in the genetic maps of 168, *trpE26* and *trpE30* types of strains. The chromosome is divided into sections within which the order of markers is the same in all three types. O and T are the origin and terminus of replication. Terminal markers of the sections are: A, *O-cysB*; B, *thr-trpE(a)*; C, *trpE(b)-ilvA*; D, *citK-T*; Ia, *O-dal*; Ib, *purB-tre*; II, *thiA-T*. The numbers on the map of 168 are the rough percentages of the total chromosome length for each section. Open rectangles correspond to postulated sequences of intrachromosomal homology of no. 3 kind. The arrows show the orientation of the segments when the 168 map is read clockwise from the origin, according to Riley & Anilionis (1978). For a detailed map of strain 168 see Henner & Hoch (1980) and for the location on the sections of the markers used in this work see Fig. 2.

strains agrees with two different configurations (Fig. 1). The experimental data are in favour of the view that transition from one to the other map is possible by recombination between the two inverted repeats of Ib.

The formation of the *trpE30* strain was interpreted by the following model: there was congression of two transforming DNA segments from the *trpE26* donor which both interacted with two chromosomes of the 168 recipient carrying *aroB2*. One segment brought the *Ib.C* heterologous junction ('novel joint') of the donor while the other transformed to *aroB*⁺ and recombined at a zone between B and D with an homologous zone existing between Ia and Ib. This recombination event created the *B.Ib* junction characteristic of the *trpE30* strain. Depending on whether the two donor segments, when integrated into the recipient genome, had the same or an inverted orientation in relation to each other, a cell with respectively map 2 or map 1 configuration was generated (Schneider & Anagnostopoulos, 1981). This model therefore implies that in the *trpE30* strains a DNA sequence exists in several regions of the chromosome which will allow this recombination to occur. The presence of three different kinds of homologous sequences (nos 1, 2 and 3) arranged in a certain pattern along the *B. subtilis* chromosome was postulated (Anagnostopoulos, 1977; Schneider *et al.* 1982). In the case of the *trpE30* strains, copies of only sequence no. 3 are thought to be involved. These are located at the following junctions of chromosome segments in the *trpE30* strains themselves: *Ia.Ib*, *B.Ib*, *C.D* and *A.B* (Fig. 1). Zones of intrachromosomal homology nos 1 and 2 seem to be implicated in the transmission of the two rearrangements (translocation and inversion) of the *trpE26* map configuration (Anagnostopoulos, 1977).

Table 1. Strains of *Bacillus subtilis* used

Strain no.	Genotype	Source* or reference
168M	<i>trpC2</i>	Laboratory stock
H25	<i>hisH2</i>	Laboratory stock
CU156	<i>trpC2 ilvA3 citD2</i>	S. A. Zahler
GSY110	<i>tyrA1</i>	Laboratory stock
GSY266	<i>metB3</i>	Laboratory stock
GSY392	<i>lys-1 aroB2 hisH2</i>	Laboratory stock
GSY1835	<i>trpE30</i>	Schneider & Anagnostopoulos (1981)
GSY1836	<i>trpE30 ilvA1 strA</i>	Schneider & Anagnostopoulos (1981)
GSY2188	<i>trpE30</i> ⁺ haploid	GSY392 → GSY1835 (Tf)
GSY2189	<i>trpE30</i> ⁺ <i>hisH2 tre-12 strA</i>	WT → GSY2185 (Tf)
GSY2197	<i>trpE30 hisH2 thrA5 tre-12/tre</i> ⁺ <i>strA</i>	Schneider & Anagnostopoulos (1981)
GSY2200	<i>trpE30 hisH2 purB34 tre</i> ⁺ / <i>purB</i> ⁺ <i>tre-12 strA</i>	Schneider & Anagnostopoulos (1981)
GSY2501	<i>trpE30 ilvA1 purB34 tre-12/purB34 tre-12 strA</i>	GSY273 → GSY2395 (Tf)
GSY2502	<i>trpE30</i> ⁺ <i>hisH2 ilvA1/hisH</i> ⁺ <i>ilvA1 purB34 tre-12/purB34 tre-12 strA</i>	H25 → GSY2501 (Tf)
GSY2503	<i>trpE30</i> ⁺ <i>hisH2/hisH</i> ⁺	GSY392 → GSY1835 (Tf)
GSY2507	<i>trpE30 metB3 strA</i>	GSY266 → GSY1836 (Td)
GSY2508	<i>trpE30</i> ⁺ <i>hisH2/hisH</i> ⁺ <i>citK7 strA</i>	H25 → GSY2186 (Tf)
GSY2510	<i>trpE30 ilvA3 citD2 strA</i>	CU156 → GSY2507 (Td)
GSY2511	<i>hisH2 citD2</i>	H25 → CU156 (Td)
GSY2512	<i>trpE8 ilvA1</i>	T8 → GSY111 (Tf)

* For strains constructed during this work, the method by which they were obtained [transformation (Tf) or PBS-1 transduction (Td)] and the parent strains are indicated; the arrows point to the recipients.

The *trpE30* strains are very stable in the sense that they never spontaneously lose the Ib duplication. Recombination events between the two copies do however occur, with reassortment of alleles in the case of clones heterozygous in this area. Rare auxotrophic cells also appear from the heterozygous clones but these are not true segregants: they are homozygous for the mutant alleles of the Ib region and derive either from recombination between two daughter chromosomes or by a mechanism of gene conversion.

It was shown that the great majority of the *trpE30*⁺ transformants and transductants were haploid cells which had lost the duplication of the Ib segment. This was explained by the hypothesis that the donor DNA segment which brings the intact *trpE*⁺ locus bridges segments B and C of either map configuration 1 or map configuration 2 and leads to the formation of cells of 168-type structure. However, considering the case of the *trpE26*⁺ transformants and transductants which are nearly all unstable merodiploids readily segregating Trp⁻ cells, we thought that some *trpE30*⁺ merodiploids might be produced. As was stated in the above mentioned paper, such clones were indeed found in small proportions. The present paper deals with their isolation, the determination of their genetic maps, the mechanism of their formation and the patterns of their segregation. These studies revealed the induction of novel rearrangements and chromosome instabilities in *B. subtilis*.

METHODS

Bacterial strains. The *B. subtilis* strains used are listed in Table 1. The *trpE30*⁺ merodiploids were selected as Trp⁺ transformants segregating His⁻ cells.

Media and procedures. Media, DNA preparation, procedures for transformation and PBS-1 mediated transduction, scoring of transformants and transductants, and search for segregants were described in previous papers (Anagnostopoulos & Spizizen, 1961; Audit & Anagnostopoulos, 1972; Jamet & Anagnostopoulos, 1969; Schneider & Anagnostopoulos, 1981; Schneider *et al.*, 1982).

RESULTS

Production of trpE30⁺ transformants and transductants carrying a duplication of the C segment

The usual procedure to detect duplications was applied to *trpE30* recipients. Transformation and transduction crosses were performed involving the *trpE30* marker and at least one other

Table 2. Presence of the *hisH2* marker in the DNA of two unstable *Trp*⁺ *His*⁺ clones from strain GSY1835 (*trpE30*) transformed by GSY392 (*aroB2hisH2*)The recipient in these crosses was 168M (*trpC2*).

Cross	Donor	Trp ⁺ transformants			Recombination (%)
		Sample	Class	No.	
1	Clone 3 Trp ⁺ Aro ⁺ His ⁺ unstable	269	Aro ⁺ His ⁺	146	<i>trpC2, hisH2</i> = 54.3
			Aro ⁺ His ⁻	123	
			Aro ⁻ His ⁺	0	
			Aro ⁻ His ⁻	0	
2	Clone 13 Trp ⁺ Aro ⁻ His ⁺ unstable	540	Aro ⁺ His ⁺	90	<i>trpC2, hisH2</i> = 51.1 <i>trpC2, aroB2</i> = 30.4
			Aro ⁺ His ⁻	74	
			Aro ⁻ His ⁺	186	
			Aro ⁻ His ⁻	190	
3	Clone 20 Trp ⁺ Aro ⁻ His ⁺ stable	736	Aro ⁺ His ⁺	137	<i>trpC2, aroB2</i> = 18.6
			Aro ⁺ His ⁻	0	
			Aro ⁻ His ⁺	599	
			Aro ⁻ His ⁻	0	
4	Clone 16 Trp ⁺ Aro ⁻ His ⁺ stable	457	Aro ⁺ His ⁺	120	<i>trpC2, aroB2</i> = 26.2
			Aro ⁺ His ⁻	0	
			Aro ⁻ His ⁺	337	
			Aro ⁻ His ⁻	0	

marker linked to the *trp* region. The possibility of segregation of auxotrophs was examined in the relevant prototrophs and the presence of the mutant allele in their DNA was tested.

In a typical experiment, strain GSY1835 (*trpE30*) was transformed by DNA (0.04 µg ml⁻¹) from strain GSY392 (*aroB2 hisH2*) of 168 type. There was high frequency of cotransfer of *trpE*⁺, *aroB2* and *hisH2* (Table 3, cross 4 of Schneider & Anagnostopoulos, 1981). We looked for the presence of any merodiploid cells in a sample of 30 Trp⁺ clones: 10 Aro⁺ His⁺, 10 Aro⁺ His⁻ and 10 Aro⁻ His⁺. The possibility of segregation of auxotrophs from these clones was first examined. In three Aro⁺ His⁺ and three Aro⁻ His⁺ clones, bacteria with a His⁻ phenotype were found, ranging from 9 to 27% of the viable counts. The 20 Aro⁺ clones studied did not segregate any Aro⁻ cells and none of the 30 clones produced any Trp⁻ bacteria. These results were confirmed by extracting DNA from each of the 30 clones and using it to transform strain 168M (*trpC2*). The three unstable Trp⁺ Aro⁺ His⁺ clones gave nearly identical results showing the presence of the *hisH2* allele in their DNA. The results with only one of them are shown in Table 2 (cross 1). DNA from the seven Trp⁺ Aro⁺ His⁺ stable clones did not produce any His⁻ or Aro⁻ transformants. Cross 2 of Table 2 is an example of the results obtained with DNA from the three Trp⁺ Aro⁻ His⁺ unstable clones. DNA from six stable Trp⁺ Aro⁻ His⁺ clones behaved like that of an *aroB2* strain of 168 type, showing a mean value of recombination frequency between *aroB2* and *trpC2* of 17% (an example is cross 3, Table 2). Only one clone (no. 16) of this stable Trp⁺ Aro⁻ His⁺ class gave a recombination frequency rather higher (Table 2, cross 4) and might be different from the other six. Finally, the DNAs from the 10 Trp⁺ Aro⁺ His⁻ stable clones did not produce any Aro⁻ transformants. They behaved like the DNA from strain H25 (*hisH2*), with a mean value of recombination frequency between *trpC2* and *hisH2* of 36.6%.

The above experiments show that some of the Trp⁺ transformants from strain GSY1835 possess a duplication of the *hisH* locus, which is situated on the C chromosome segment. Their *aroB* locus, situated on segment B, is not duplicated. There is evidence that the duplication also involves the *trpC* locus. The proportion of Trp⁺ His⁺ recombinants is higher with the six heterozygous *hisH2/hisH*⁺ clones as donors for transforming 168M (*trpC2*), 54.2%, than with the 10 haploid *hisH2* ones, 36.6%. The increase can be interpreted by the presence in the heterozygous donors of two types of DNA segments able to transform *trpC2*: *aroB*⁺ (or *aroB2*) *trpC*⁺ *hisH2* and *trpC*⁺ *hisH*⁺. The presence of these two segments also results in an increase in the recombination frequency between *trpC2* and *aroB2*. It has a mean value of 31.7% with the three Aro⁻ His⁺ segregating clones while this value is of 17.0% with the six stable clones

Table 3. Production of *trpE30*⁺ transductants possessing duplications of the *hisH* and *ilvA* loci

Cross	Recipient	Donor	Transductants				
			Selection	Sample	Class	No.	%
1	GSY1836 <i>trpE30 ilvA1</i>	H25 <i>hisH2</i>	Trp ⁺ †	1977	His ⁺ Ile ⁺	72	3.6
					His ⁺ Ile ⁻	48	2.4
					His ⁻ Ile ⁺	918	46.5
					His ⁻ Ile ⁻	939	47.5
			Ile ⁺	926	Trp ⁺ His ⁺	69	7.4
					Trp ⁻ His ⁺	446	48.2
					Trp ⁺ His ⁻	410	44.3
					Trp ⁻ His ⁻	1	0.1
2*	GSY2512 <i>trpE8 ilvA1</i>	H25 <i>hisH2</i>	Trp ⁺ ‡	656	His ⁺ Ile ⁺	4	0.6
					His ⁺ Ile ⁻	11	1.7
					His ⁻ Ile ⁺	311	47.4
					His ⁻ Ile ⁻	330	50.3
			Ile ⁺	603	Trp ⁺ His ⁺	3	0.5
					Trp ⁻ His ⁺	296	49.0
					Trp ⁺ His ⁻	294	48.8
					Trp ⁻ His ⁻	10	1.7

* Control experiment with both donor and recipient of 168 type.

† Ratio of transductants obtained per ml Trp⁺/Ile⁺ = 0.67.

‡ Ratio of transductants obtained per ml Trp⁺/Ile⁺ = 1.27.

of the same phenotype. The rather high recombination measured with clone no. 16 (Table 2, cross 4) suggests that it may not be haploid like the other six but homodiploid *hisH*⁺/*hisH*⁺.

The proportion of heterozygous *hisH2*/*hisH*⁺ clones among the Trp⁺His⁺ transformants of strain GSY1835 in the above experiment was therefore 30%. Taking into account the relative proportions of the classes of Trp⁺ transformants where these heterozygous clones were found (Table 3, cross 4 of Schneider & Anagnostopoulos, 1981), it can easily be calculated that the clones carrying a duplication of the *trpC*-*hisH* region were about 10% of the total. A similar experiment gave identical results. The recipient was GSY1836 (*trpE30 ilvA1*) and the donor DNA came from strain H25 (*hisH2*). Among the Trp⁺ transformants 9% segregated His⁻ cells; these made up one-third of the Trp⁺His⁺Ile⁻ class.

To determine whether *trpE30*⁺ clones carrying a duplication are also produced during PBS-1 mediated transduction and whether the duplication covers the entire C segment, strain GSY1838 (*trpE30 ilvA1*) was transduced by a lysate from H25 (*hisH2*) (Table 3, cross 1). The *ilvA1* marker is located at one end of the C segment and linked in transduction to *hisH2* with cotransfer frequencies of about 48%, as indicated in the control experiment (Table 3, cross 2) where both recipient, GSY2512 (*trpE8 ilvA1*), and donor, H25, were 168-type strains. The *hisH2* marker is very strongly linked in transduction to the *trpE* locus (cross 2). Comparison of the results of the two crosses reveals the following: (a) the ratio of the Trp⁺/Ile⁺ transductants from the GSY1836 recipient is half that of the control cross (this is due to the difficulty of integration of the *trpE30*⁺ marker); (b) the proportion of prototrophs Trp⁺His⁺Ile⁺ among both the Trp⁺ and the Ile⁺ transductants is much higher in cross 1 than in cross 2. This suggests that some of them, in cross 1, may be heterozygous for *hisH* and *ilvA*.

To test this assumption we studied the segregation of 24 Trp⁺His⁺Ile⁺ clones. All these were found to be merodiploid *trpE30*⁺*hisH2 ilvA*⁺/*hisH*⁺*ilvA1*: the cultures of each clone contained from 11 to 20% auxotrophs. The mean values of the proportions of the various phenotypic classes were: His⁻Ile⁻ (48.7%), His⁻Ile⁺ (48.6%) and His⁺Ile⁻ (2.7%). No Trp⁻ segregants were found. The 48 Trp⁺His⁺Ile⁻ clones of cross 1 were analysed for the presence of the *hisH2* marker by using their DNA to transform strain 168M. Eight of them did in fact possess this marker (results as cross 1, Table 2) and their genotype must be *trpE30*⁺*hisH2 ilvA1*/*hisH*⁺*ilvA1*. If we suppose that all Trp⁺His⁺Ile⁺ transductants were merodiploid we can conclude that in this experiment about 4% of the Trp⁺ transductants were merodiploid, a lower figure than that

obtained in the same cross performed by transformation (see above). It is noticeable that the great majority of the merodiploid transductants carry both markers of the donor (*hisH2* and *ilvA*⁺). Considering the known recombination frequency between *hisH2* and *ilvA1* in transduction, one would expect to obtain equal proportions of Ile⁺ and Ile⁻ *trpE30*⁺ merodiploid transductants. The above results suggest the possibility of a difference in the mechanism of production of the two classes of these merodiploids.

Another similar experiment was performed by transducing the same recipient strain GSY1836 by a lysate of GSY266 (*metB3*). The *metB3* marker is located between *trpE* and *ilvA1* and linked to the latter in transduction with a cotransfer frequency of 80%. Out of 10 Trp⁺ Met⁺ Ile⁺ transductants studied, nine readily produced auxotrophs of the following phenotypes: Met⁻ Ile⁺ (54%), Met⁺ Ile⁻ (37%) and Met⁻ Ile⁻ (9%). Conversely, 70 Trp⁻ Met⁺ Ile⁺ clones did not produce any segregants.

Since the above studies demonstrated duplication of the *trpC*, *hisH*, *metB* and *ilvA* loci in *trpE30* recipients, we can conclude that introduction by either transformation or transduction of the intact *trpE*⁺ locus in those strains sometimes induces duplication of segment C. The fact that no segregation was observed in Trp⁻ transductants for other markers of segment C implies that induction of a duplication in this area cannot take place unless the *trpE30* marker is transformed or transduced.

Genetic map(s) of the trpE30⁺ merodiploids

The high frequency of segregation in the *trpE30*⁺ clones, heterozygous for markers of segment C, suggests that either the duplication of this segment is tandem or that the two copies of C are separated by one of the Ib copies of the non-tandem duplication of the *trpE30* strains. In the latter case, recombination between the two copies of C would eliminate one copy of Ib. To test this hypothesis we chose as recipient a *trpE30* strain heterozygous for two markers of segment Ib. Strain GSY2200 (*trpE30 hisH2 purB34 tre*⁺/*purB*⁺ *tre-12*) was transformed by DNA from GSY110 (*tyrA1*). *tyrA1* lies on segment C and is cotransformed with *hisH2* with a frequency of about 82%. Trp⁺ His⁺ Tyr⁺ transformants were selected and their phenotype as regards adenine requirement and trehalose utilization was determined. Among 553 Trp⁺ His⁺ Tyr⁺ colonies we found 55.3% Ade⁺ Tre⁺, 34.5% Ade⁻ Tre⁺, 5.6% Ade⁺ Tre⁻ and 4.5% Ade⁻ Tre⁻. We then proceeded to study the segregation of 18 Trp⁺ His⁺ Tyr⁺ Ade⁺ Tre⁺ clones (Table 4). Only one (no. 7) was stable and was most probably haploid. The remaining 17 all segregated auxotrophic bacteria with various combinations of markers. The proportions of auxotrophs in the cultures varied from 5.5% to 47%. The majority (12 clones) segregated Tyr⁻ cells. They should therefore be *trpE30*⁺ *hisH*⁺ *tyrA1*/*hisH2 tyrA*⁺. Those that did not segregate Tyr⁻ (nos 2, 3, 5, 9 and 10) could be *trpE30*⁺ *hisH*⁺ *tyrA*⁺/*hisH2 tyrA*⁺. The fact that Ade⁻ and Tre⁻ segregants appeared shows that the duplication of the Ib segment of the recipient is retained in the *trpE30*⁺ merodiploids for markers of segment C and indicates that haploidization for this segment concomitantly eliminates one of the copies of segment Ib as postulated above. The prevailing phenotypic class for the markers of segment Ib among the segregants was not however the same for all clones analysed: 11 clones gave mainly or uniquely Ade⁻ Tre⁺, three clones (nos 12, 14 and 16) Ade⁺ Tre⁻, one clone (no. 4) Ade⁺ Tre⁺, and two clones (nos 15 and 17) Ade⁺ Tre⁺, Ade⁻ Tre⁺ and Ade⁻ Tre⁻ in almost equal numbers. This rather complex segregation pattern will be considered in the Discussion. What is already excluded is the possibility of a tandem duplication for segment C in the *trpE30*⁺ merodiploids. In view of the fact that two genetic maps are possible for the *trpE30* strains, more than one configuration could be predicted for the duplication of segment C. We first considered one possibility: B.C.Ib.C.D.

In order to obtain direct information on the genetic map of the *trpE30*⁺ merodiploids, linkage studies in transduction were carried out for markers at the C.Ib, Ib.C and C.D junctions. Cross 1 of Table 5 shows that the C.Ib junction is present in the merodiploids since *ilvA1* is linked to *purB34*. The linkage value (about 6%) was underestimated because both donor and recipient possessed two copies of the *ilvA* and *purB* loci but only one *ilvA*-*purB* linkage. Moreover, the presence in the donor of haploid segregants which have lost the junction added to the decrease in cotransfer frequency of *ilvA*⁺ and *purB*⁺. The first control cross (no. 2) provides evidence that

Table 4. Segregation of 18 *Trp*⁺ *His*⁺ *Tyr*⁺ *Ade*⁺ *Tre*⁺ clones from strain GSY2200 transformed by DNA from GSY110 (*tyrA1*)

Clone no.	Sample of colonies examined	Phenotype of segregants				No.	Clone no.	Sample of colonies examined	Phenotype of segregants				No.
		His	Tyr	Ade	Tre				His	Tyr	Ade	Tre	
1	168	+	-	-	+	27	13	147	+	-	-	+	46
		+	+	+	-	1			+	-	+	+	1
2	94	+	+	-	+	31			+	+	-	-	1
3	290	-	+	-	+	80	14	219	+	-	+	-	13
		-	+	+	+	2			+	-	-	+	7
		+	+	-	+	7			+	-	-	-	1
		+	+	+	-	1			+	+	+	-	4
4	132	+	-	+	+	21			-	+	+	+	2
		+	+	-	+	1			-	+	-	+	1
5	160	+	+	-	+	13	15	208	+	-	+	+	6
		+	+	-	-	3			+	-	-	-	6
		+	+	+	-	1			+	-	-	+	3
		-	+	+	-	2			+	-	+	-	1
6	130	+	-	-	+	13	16	138	+	-	+	-	12
									+	-	+	+	9
7	264					0			+	-	-	-	3
8	88	+	-	-	+	7			+	+	-	+	1
		+	+	-	+	2			+	+	+	-	1
9	109	+	+	-	+	5			+	+	-	-	1
		+	+	+	-	1	17	195	+	-	-	+	34
10	157	+	+	-	+	43			+	-	-	-	29
11	131	+	-	-	+	22			+	-	+	+	26
		+	-	+	+	1			+	-	+	-	1
		+	+	+	-	1			+	+	-	+	1
12	134	+	-	+	-	56	18	237	+	-	-	+	32
		+	-	-	-	1			+	-	+	-	2
		+	+	+	-	2			+	-	-	-	2
		-	+	+	-	3			+	+	+	-	2

haploid *trpE30*⁺ strains do not possess the $\text{C} \cdot \text{Ib}$ junction. The second control cross (no. 3) shows that a *trpE30* strain, when used as donor, reveals a very weak linkage of *ilvA1* to *purB34* (0.09%). The significance of this point will be considered in the Discussion.

The presence of the $\text{Ib} \cdot \text{C}$ junction in the *trpE30*⁺ merodiploids is shown in cross 4 of Table 5. The recipient (GSY2197) is a *trpE30* strain heterozygous *tre-12/tre*⁺ and possessing *hisH2* linked to *tre*⁺ (Schneider & Anagnostopoulos, 1981). The measured cotransfer frequency between *hisH*⁺ and *tre-12* markers of the donor merodiploid (about 40%) is almost the same as in a *trpE30* strain (Schneider & Anagnostopoulos, 1981). This cross has also shown that (a) the *hisH* and *ilvA* loci were normally linked in the *trpE30*⁺ merodiploid, and (b) the *hisH*⁺ allele of this merodiploid was unlinked to *trpE30*⁺. The control cross (no. 5) proved that *trpE30*⁺ haploid clones did not possess the *hisH-tre* linkage ($\text{Ib} \cdot \text{C}$ junction).

The existence of the $\text{C} \cdot \text{D}$ junction was studied in a repulsion cross between two merodiploids involving markers *ilvA1* and *citK7* (Table 5, cross 6). Among the *Ile*⁺ transductants about 10% were *Cit*⁻. The conclusion was that the *ilvA* and *citK* loci were linked and that the merodiploid *trpE30*⁺ were haploid for *citK*. Were this locus duplicated the integration of the mutant allele *citK7* would not be detectable. The transduced culture was plated without added histidine. The *His*⁻ segregants of the recipient were therefore counterselected. To eliminate the hypothesis that the *Cit*⁻ transductants came from *His*⁺ haploid segregants we studied the segregation of the 20 *Cit*⁻ clones. They all readily segregated auxotrophic cells. According to their segregation pattern they fall into two distinct groups. In 17 clones the mean relative proportions of the auxotrophic cells were the following: *His*⁻ *Ile*⁺ (99.2%), *His*⁺ *Ile*⁻ (0.3%) and *His*⁻ *Ile*⁻ (0.5%). In the second

Table 5. *Linkage relationships in transduction of the following pairs of loci in the trpE30⁺ haploid and merodiploid strains: purB-ilvA, hisH-tre and ilvA-citK*

Cross	Recipient	Donor	Transductants				Cotransduction (%)
			Selection	Sample	Class	No.	
1	GSY2502 <i>trpE30⁺</i> <i>hisH2 ilvA1/hisH⁺ ilvA1</i> <i>purB34/purB34</i>	GSY2503 <i>trpE30⁺</i> <i>hisH2/hisH⁺</i>	Ile ⁺	1602	Ade ⁺	106	<i>purB34,ilvA1</i> = 6.6
					Ade ⁻	1496	
			Ade ⁺	2389	Ile ⁺	133	<i>purB34,ilvA1</i> = 5.6
					Ile ⁻	2256	
2	GSY2502 <i>trpE30⁺</i> <i>hisH2 ilvA1/hisH⁺ ilvA1</i> <i>purB34/purB34</i>	GSY2188 <i>trpE30⁺</i> (haploid)	Ile ⁺	1772	Ade ⁺	0	<i>purB34,ilvA1</i> = 0.0
					Ade ⁻	1772	
			Ade ⁺	1123	Ile ⁺	0	<i>purB34,ilvA1</i> = 0.0
					Ile ⁻	1123	
3	GSY2502 <i>trpE30⁺</i> <i>hisH2 ilvA1/hisH⁺ ilvA1</i> <i>purB34/purB34</i>	GSY1835 <i>trpE30</i>	Ade ⁺	2195	Ile ⁺	2	<i>purB34,ilvA1</i> = 0.09
					Ile ⁻	2193	
4	GSY2197 <i>trpE30</i> <i>hisH2</i> <i>tre⁺/tre-12</i>	GSY2502 <i>trpE30⁺</i> <i>hisH2 ilvA1/</i> <i>hisH⁺ ilvA1</i> <i>tre-12/tre-12</i>	His ⁺	199	Trp ⁻ Tre ⁺ Ile ⁺	84	<i>hisH2,tre-12</i> = 39.9
					Trp ⁻ Tre ⁻ Ile ⁺	64	<i>hisH2,ilvA1</i> = 25.3
					Trp ⁻ Tre ⁺ Ile ⁻	35	
					Trp ⁻ Tre ⁻ Ile ⁻	15	
					Trp ⁺ Tre ⁻ Ile ⁻	1	
5	GSY2189 <i>trpE30⁺</i> <i>hisH2 tre-12</i> (haploid)	GSY2188 <i>trpE30⁺</i> (haploid)	His ⁺	6507	Tre ⁺	0	<i>hisH2,tre-12</i> = 0.0
					Tre ⁻	6507	<i>hisH2,tre-12</i> = 0.0
			Tre ⁺	2182	His ⁺	0	
					His ⁻	2182	
6	GSY2502 <i>trpE30⁺</i> <i>hisH2 ilvA1/hisH⁺ ilvA1</i>	GSY2508 <i>trpE30⁺</i> <i>hisH2/hisH⁺</i> <i>citK7</i>	Ile ⁺	200	Cit ⁺	180	<i>ilvA1,citK7</i> = 10.0
					Cit ⁻	20	
7	GSY2508 <i>trpE30⁺</i> <i>hisH2/hisH⁺ citK7</i>	GSY2502 <i>trpE30⁺</i> <i>hisH2 ilvA1/</i> <i>hisH⁺ ilvA1</i>	Cit ⁺	1580	Ile ⁺	1580	<i>ilvA1,citK7</i> = 0.0
					Ile ⁻	0	

group, of three clones, the mean frequencies of these classes were: His⁻ Ile⁺ (55.9%), His⁺ Ile⁻ (1.7%) and His⁻ Ile⁻ (42.4%). These results suggested that the *trpE30⁺* merodiploids were not all identical in their genetic structure. This point will be discussed below. The cotransfer frequency of *ilvA⁺* and *citK7* measured in cross 6 (Table 5) was underestimated because of the presence of two *ilvA* loci in the donor and in the recipient, only one of which was linked to *citK*. In the *trpE30*-type strains the cotransduction frequency of *ilvA1* and *citK7* (about 15%) is identical with that of the 168-type strains (Schneider & Anagnostopoulos, 1981). Cross 7 of Table 5 is the reciprocal of cross 6; it demonstrated the duplication of the *ilvA* locus in strain GSY2508. This Cit⁻ strain which segregates His⁻ cells is therefore diploid for the whole C segment.

From these results on the merodiploids studied it appears that (a) they carry a non-tandem duplication of the whole of segment C which does not seem to extend into segment D, and a duplication of segment Ib; and (b) they possess the $\overline{C}$.Ib, $\overline{Ib}$. $\overline{C}$ and $\overline{C}$. $\overline{D}$ junctions. Because of the length of the duplicated areas, these results do not allow one to determine the spatial relationships of the two copies of these areas, i.e. to construct a unique chromosomal map. Such was also the case for the *trpE30* strains for which two maps are possible (Fig. 1). For those clones that possess a duplication of both segments Ib and C we propose three maps which fit all the results obtained (Fig. 2). Recombination is possible between copies of the duplicated segments. A single reciprocal recombination event between inverted repeats of either segment Ib or segment C is sufficient to make the transition from map *a* to map *b*, from map *b* to map *c* and vice versa. The segregation patterns (also shown in Fig. 2) may help in deciding as to the prevailing map configuration in a particular clone. These points will be further dealt with in the Discussion.

Fig. 2. Proposed genetic maps and haploidization patterns of the *trpE30*⁺ transformants and transductants carrying non-tandem duplications of both segment 1b (*purB-tre*) and segment C (*trpE(b)-ilvA*). The sections of the maps are not drawn to scale. For the segregation step of maps *a* and *c*, only the relevant part of the chromosome is shown. For more details on the presentation of the maps see Fig. 1.

Studies on the nature of the homologous sequences

It was stated in the Introduction and in previous papers that homologous sequences, not all identical, are present at different points of the chromosome (Fig. 1) and may play a role in many of the observed rearrangements. The hypothesis was considered that the copies of the sequences involved in the formation of the *trpE30* strains (sequences no. 3) were identical with the prophage SP β or a part of it (Schneider & Anagnostopoulos, 1981). All strains of 168 type are lysogenic for this phage, which was discovered by Warner *et al.* (1977) and studied by Zahler and coworkers (Zahler *et al.*, 1977, 1982; Rosenthal *et al.*, 1979; Fink *et al.*, 1981). SP β is inserted into the chromosome of 168-type strains between *ilvA* and *kauA*, i.e. at the C₁D₁ junction. It can, however, attach itself to other regions, one of which is near the *dal* locus (Lipsky *et al.*, 1981). This position may be identical with the I_aI_b junction. The models elaborated for interpreting the production of the *trpE30*⁺ merodiploids, which will be developed in the Discussion, imply the participation of homologous sequences no. 3 in these events (Fig. 3). In order to test the possibility of the involvement of phage SP β in the above case, we carried out experiments with strains possessing the marker *citD2*, which corresponds to a deletion of SP β or at any rate the greater part of it. The deletion includes also the *kauA* and *citK* loci and thus confers a Cit⁻ phenotype (Zahler *et al.*, 1982). The first cross of Table 6 shows the results of the transduction of a *trpE30* strain carrying the *citD2* deletion by a lysate from strain H25 (*hisH2*, 168-type, lysogenic). In the second cross both donor and recipient possessed *citD2*. Cross 1 of Table 3 (Trp⁺ selection) was the corresponding control experiment. In all three crosses the His⁺ Ile⁻ class

Fig. 3. Models for the formation of merodiploid *Trp*⁺ transformants and transductants from a *trpE30* strain. The donor DNA segment (represented by a thin line) interacts with two chromosomes of the recipient (shown in black and white). For models A and B only part of the first chromosome is drawn. The homologous sequences at the C-D and B-Ib junctions are represented as hatched rectangles. The recipient chromosomes are of either map 1 (model A) or map 2 (models B and C) configuration. Dotted lines represent recombination events. On the maps of the clones formed the four homologous zones no. 3 are shown.

Table 6. Effect of the *citD2* deletion on the production of *trpE30*⁺ merodiploids in transduction

Cross	Recipient	Donor	Trp ⁺ transductants			
			Sample	Class	No.	%
1	GSY2510 <i>trpE30 ilvA3 citD2</i>	H25 <i>hisH2</i>	1877	His ⁺ Ile ⁺	364*	19.4
				His ⁺ Ile ⁻	24*	1.3
				His ⁻ Ile ⁺	210	11.2
				His ⁻ Ile ⁻	1289	68.7
2	GSY2510 <i>trpE30 ilvA3 citD2</i>	GSY2511 <i>hisH2 citD2</i>	1549	His ⁺ Ile ⁺	2	0.1
				His ⁺ Ile ⁻	34	2.2
				His ⁻ Ile ⁺	850	54.9
				His ⁻ Ile ⁻	663	42.8

* 68 of the 364 His⁺ Ile⁺ and all the 24 His⁺ Ile⁻ transductants were tested for the Cit character: all were Cit⁻

was about 2% of the Trp⁺ transductants. It was shown in the control cross that eight clones among 48 of this class were merodiploid. In cross 1 of Table 6 we looked for the presence of the *hisH2* allele in the DNA of the 24 His⁺ Ile⁻ clones: none of them transmitted this allele to strain 168M. They were therefore haploid. The extremely high number of the His⁺ Ile⁺ clones found in cross 1 (Table 6) as compared to cross 2, suggested that this class may be composed of merodiploids. This was confirmed after studying the segregation of 14 such clones of cross 1. In all cultures auxotrophic cells were found ranging from 2 to 6% of the viable counts. The mean values of the relative proportions of the various phenotypic classes were: His⁻ Ile⁻ (63.8%), His⁻ Ile⁺ (29.9%) and His⁺ Ile⁻ (6.3%). Conversely, the two His⁺ Ile⁺ clones of cross 2 proved to be haploid: their DNA did not transfer *hisH2* into 168M.

Similar results were obtained by transformation. DNA from strain H25 (*hisH2*) was used at limiting concentration (0.01 µg ml⁻¹) to transform strain GSY2510 (*trpE30 ilvA3 citD2*). The

Trp⁺ His⁺ Ile⁻ class was 31.2% of the Trp⁺ transformants. Thirty clones from this class were analysed. Not one clone of this sample segregated His⁻ bacteria. When the recipient was strain GSY1836, one-third of the Trp⁺ His⁺ Ile⁻ transformants were merodiploid (see first section of Results). These results can be summarized as follows: (a) absence of Trp⁺ His⁺ Ile⁻ merodiploid clones in transduction and transformation when the recipient carried *citD2*; (b) absence of Trp⁺ His⁺ Ile⁺ merodiploid transductants on condition that both donor and recipient carried the deletion. The production of merodiploid Trp⁺ His⁺ Ile⁺ transductants when the donor did not carry the deletion will be interpreted below. These facts indicate that the *citD2* deletion hinders the formation of the *trpE30*⁺ merodiploids.

It is of interest to note that lack of homology between donor and recipient in the vicinity of the *ilvA* locus (cross 1, Table 6) greatly reduced the cotransduction frequency of *trpE30*⁺ with *ilvA3*⁺ during formation of haploid transductants (13.8%, instead of 55.0% as in cross 2).

DISCUSSION

A glance at the maps in Fig. 2 reveals the following features. In map *a* the chromosome possesses two inverted copies of segment Ib, while in map *b* the inverted repeats involve both segment Ib and segment C, and in map *c* only segment C. As stated above (Results), single recombination events lead from map *a* to map *b* and then to map *c*, and vice versa. It is possible that, as in the case of the *trpE30* strains (transition between maps 1 and 2: Schneider & Anagnostopoulos, 1981), the cultures of the *trpE30*⁺ merodiploids are heterogeneous and contain cells with chromosomes of all three configurations.

As regards the segregation of these merodiploids, although they all yield haploid cells of 168 type, this could happen in different ways according to the map configuration of the cell. In the chromosome of map *a* a single recombination between the two straight repeats of segment C excises one copy of both segment Ib and segment C. The segregation patterns of 24 Trp⁺ His⁺ Ile⁺ and nine Trp⁺ Met⁺ Ile⁺ transductants of strain GSY1836 (first section of Results) imply such a recombination event inside segment C. Their genotypes therefore should be mainly *trpE30*⁺ *hisH2* *ilvA*⁺/Ib/*hisH*⁺ *ilvA1* and *trpE30*⁺ *metB3* *ilvA*⁺/Ib/*metB*⁺ *ilvA1* respectively. The chromosome of three out of 20 Cit⁻ clones of cross 6, Table 5 (those which segregate mainly His⁻ Ile⁺ and His⁻ Ile⁻ cells) must also have a map *a* configuration: *trpE30*⁺ *hisH2* *ilvA1*/Ib/*hisH*⁺ *ilvA*⁺ *citK7*. The relative proportions of the segregant classes of all these clones suggest that the distance between *ilvA* and the end of the homology is about the same as that between *trpE30*⁺ and *ilvA*. When the *trpE30*⁺ clones are heterozygous for markers of segment Ib, this segregation pattern will retain the markers of Ib located on the copy of this segment flanked by Ia and II. This is clearly the case for clone nos 1, 2, 3, 10, 11, 13 and 18 (Table 4), and also for clone nos 5, 6, 8 and 9, although the number of segregants obtained was lower. They did yield a majority of cells which had *purB34* and *tre*⁺ on this copy of Ib. However, in clones no. 4 and no. 12, this copy of Ib carried the couples *purB*⁺, *tre*⁺ and *purB*⁺, *tre-12* respectively. These clones may be the result of previous exchanges of alleles between the two Ib copies as mentioned in the previous paper (Schneider & Anagnostopoulos, 1981).

In the case of chromosomes with a map *c* configuration (Fig. 2), a recombination event between the direct repeats of segment Ib results in the loss of one copy of the Ib-C duplication. If the culture is homogeneous and the strain heterozygous for *purB* and *tre*, the segregants for the alleles of those two loci will not have a unique phenotype (as in the case of map *a*), but will reflect the different possibilities of recombination inside the Ib segment. Clone no. 17 of Table 4, which yields about equal proportions of Ade⁻ Tre⁺, Ade⁻ Tre⁻ and Ade⁺ Tre⁺ segregants, fits a map *c* configuration where the mutant alleles *purB34* and *tre-12* should be located on the Ib copy linked to Ia. On the other hand, in clones of this configuration the alleles of segment C must segregate as a whole. This was indeed found with the 17 Cit⁻ transductants of strain GSY2502 (Table 5, cross 6) which yielded 99.2% His⁻ Ile⁺ segregants. Their genotype must be *trpE30*⁺ *hisH2* *ilvA*⁺ *citK7*/II/Ib/*ilvA1* *hisH*⁺. These results suggest that the *trpE30*⁺ merodiploid clones have the *a* configuration more frequently than the *c* configuration.

As regards map *b* configuration, although the chromosome does not possess direct repeats, the

cells may yield segregants but in principle much less frequently than those of the other two types (Fig. 2).

In deciding about the genetic map of a *trpE30*⁺ merodiploid clone we considered the majority classes of its segregants. The appearance of the minority classes can easily be explained as already has been done for the *trpE30* strains themselves (Schneider & Anagnostopoulos, 1981). Two recombination events will lead to exchange of alleles without transition from one map configuration to the other. Such an exchange will be revealed in subsequent segregation by producing a minority class clone. Transition from one map to another during the culture of a clone would also give a few segregants with different marker associations. Finally, exchanges between two chromosomes may also lead to apparent segregation of auxotrophs without even loss of the duplication. These clones will nevertheless be rare.

The mechanism of production of the *trpE30*⁺ merodiploids raises several questions. It is proposed that in transformation they are formed by interaction of the exogenous DNA segment with two chromosomes of a binucleate cell or with two arms of one chromosome in the course of its replication. A third recombination event is necessary to end up with a circular merodiploid chromosome. The models we can propose for the induction of the C segment duplication in the *trpE30* strains are shown in Fig. 3. Although these are presented as formal models it was ascertained that they also hold as molecular models, specially as regards the orientation of each DNA strand. The donor DNA segment carrying the intact *trpE*⁺ locus pairs at the distal (from the origin) endpoint of the B segment of one recipient chromosome and at the proximal endpoint of segment C of the other chromosome. The third recombination event takes place between two copies of an intrachromosomal homology sequence (no. 3) located in two different parts of each of the two chromosomes: the B.Ib and the C.D junctions. If the chromosome of the *trpE30* recipient cell is of map 1 configuration the *trpE30*⁺ merodiploid will have a map *a* configuration (Fig. 3, A). If the recipient has a chromosome of map 2 configuration there are two possibilities. (1) The C.D junction of the second chromosome pairs with the B.Ib junction of the first (the one that brings its segment B to the merodiploid). In this case the merodiploid has a map *b* configuration (Fig. 3, B). (2) The C.D junction of the first chromosome pairs with the B.Ib junction of the second. In the latter case the merodiploid has a map *c* configuration (Fig. 3, C). As in their parental *trpE30* strains the *trpE30*⁺ merodiploids possess four copies of the homologous sequence no. 3: at the Ia.Ib, Ib.C (or C.Ib), D.C (or C.D) and B.A junctions.

The same models (Fig. 3) also hold for the production of *trpE30*⁺ merodiploids in transduction crosses. However, another possibility (not included in Fig. 3) may exist for the following case: when in a transduction cross the donor segment carries the intact *trpE*⁺ locus and at least part of the sequence at the C.D junction. The segment may then be inserted between regions B and Ib of a single recipient chromosome (of map 1 or map 2 configuration) if recombination takes place between the B.Ib sequence of the recipient and that of the C.D junction of the donor. The model is part of Fig. 4. In cross 1 of Table 3 the great majority of the merodiploids might have been formed in this way since about 78 of them carried the two donor markers (genotype *trpE30*⁺ *hisH2* *ilvA*⁺/*hisH*⁺ *ilvA1*) and only eight of these were recombinants (*trpE30*⁺ *hisH2* *ilvA1*/*hisH*⁺ *ilvA1*).

The experiments with strains carrying the *citD2* deletion confirm the models of Fig. 3. If the recipient *trpE30* strain does not possess a copy of sequence no. 3 at the C.D junction (which could be part of the SP β prophage), production of *trpE30*⁺ merodiploid clones is not possible unless the sequence is brought by the donor transducing segment. This was shown by the transformation cross mentioned above (third section of Results) where the donor segments are not long enough to carry the C.D junction. On the other hand, cross 1 of Table 6 shows that strain H25 (*hisH2*, SP β lysogen) could indeed form *trpE30*⁺ *hisH2* *ilvA*⁺/*hisH*⁺ *ilvA3* *citD2* clones when used as donor to transduce strain GSY2510. These *trpE30*⁺ merodiploid transductants are produced by interaction of the donor with a single recipient chromosome as shown in Fig. 4. In the same cross no *trpE30*⁺ *ilvA3*/*ilvA3* clones were found. According to the model of Fig. 4 such clones could only result from a quadruple crossover and the interaction of two chromosomes and therefore must be very rare. The absence of a no. 3 sequence at the C.D junction in the transducing segments of strain GSY2511 (*hisH2* *citD2*) in cross 2 of Table 6 does not allow the

Fig. 4. Model for the production of *trpE30*⁺ merodiploid clones during transduction of a *trpE30* strain carrying *citD2* (SPβ deleted) by a PBS-1 lysate from a 168-type strain lysogenic for SPβ. In the cross drawn the recipient was strain GSY2510 (*trpE30 ilvA3 citD2*, Map 1) and the donor H25 (*hisH2*) (Table 6, cross 1). For details on the drawing see Fig. 3. It is supposed that a sequence no. 3 is absent at the C-D junction of the recipient due to the *citD2* deletion. Merodiploids can be formed by interaction of the donor with a single recipient chromosome (crossing-overs 1 and 5). Their genotype is *trpE30*⁺ *hisH2* *ilvA*⁺/*hisH*⁺ *ilvA3 citD2*. This is a more general way of merodiploid production in transduction for all *trpE30* strains (see text). Four recombination events may also produce merodiploids but in these cases two recipient chromosomes are needed: crossing-overs 1, 2, 4 and 5 → *trpE30*⁺ *hisH*⁺ *ilvA3*/*hisH*⁺ *ilvA3 citD2*; crossing-overs 1, 3, 4 and 5 → *trpE30*⁺ *hisH2 ilvA3*/*hisH*⁺ *ilvA3 citD2*.

formation of merodiploid clones with a *trpE30 citD2* recipient. These experiments have demonstrated that the sequence of the C-D junction is always involved in the production of the segment C duplication in *trpE30* strains.

The question arises as to whether the proportion of the merodiploids among the Trp⁺ transformants of a *trpE30* strain (about 10%) is compatible with the models of Fig. 3. It is clear from the previous work on the *trpE26* strains that fusion of two chromosomes mediated by a donor DNA segment is a frequent event (Audit & Anagnostopoulos, 1972, 1975; Trowsdale & Anagnostopoulos, 1975). In the case of the *trpE30*⁺ merodiploids one has to suppose that the homologous sequences no. 3 are rather long. When describing the *citD2* deletion, Zahler *et al.* (1982) stated that it covers the greater part, if not all, of the SPβ prophage. It is true that the *c* locus of the repressor and the attachment site (*att*) are deleted. However, it is not known how far on the left side (near *ilvA*) the deletion extends. Sequence no. 3 could therefore be part either of SPβ or of the region between *ilvA* and this prophage. If SPβ was split in two in the *trpE26* strain and one part followed the original translocated segment C while the other remained at the B-D junction, then sequence no. 3 is in the distal part of SPβ (near *kauA*). The homology of all no. 3 sequences may not be complete. One can speculate that either there are pieces of SPβ scattered on the *B. subtilis* chromosome or there exist bacterial chromosomal sequences having homology for a sizable length with one (or several) part(s) of SPβ.

If recombination occurs between the homologous zones at the C-D and at the B-Ib junctions of two chromosomes during transformation or transduction of *trpE30* strains (Fig. 3), one may suppose that the same recombination events could occur in the absence of exogenous DNA. Two chromosomes of map 1 configuration fused in this manner and then recombining at any part, except in the Ib-C region, would form a merodi(tri)ploid clone: Ia.Ib.II.D.C.Ib.C.Ib.B.A. With a cell of map 2 configuration the second recombination event must take place between copies of the Ib segment of the two chromosomes. The result would be: Ia.Ib.C.Ib.II.D.C.Ib.B.A. Transformation or transduction of Trp⁺ involving a single chromosome of these clones will produce *trpE30*⁺ merodiploids of map *a* configuration in the first case and of map *b* or *c* configuration in the second, thus providing an alternative hypothesis to the proposed models of Fig. 3. One wonders whether cells with such spontaneous du(tri)-plications form a significant part of the population of the *trpE30* strains. The fact that 70 Trp⁺ Met⁺ Ile⁺ transductants of GSY1836 did not segregate any Met⁻ or Ile⁻ auxotrophs (first section of Results) offers a negative answer. This was confirmed by the very weak linkage of

purB⁺ to *ilvA*⁺ (Ib. C junction) in GSY1835 (Table 5, cross 3). If there existed in the *trpE30* strains cells already diploid for segment C they must be a negligible fraction. Interchromosomal recombination between different copies of no. 3 sequence might also spontaneously induce tandem duplications in *B. subtilis* strains of either 168 or *trpE26* type (Fig. 1), but in fact cells with spontaneous C. B tandem duplications were not found in the *trpE26* strains (Anagnostopoulos, 1977). Spontaneous tandem duplications were observed in *Escherichia coli* after mild mutagenesis by various agents (Hill *et al.*, 1977*a, b*), and also in *Salmonella typhimurium* (Anderson & Roth, 1977, 1981). The proportions of cells carrying tandem duplications in populations of these two organisms may sometimes be high (up to 10%) depending on the chromosome region considered. Most of them arise by recombination between different copies of transfer or ribosomal RNA genes belonging to two chromosomes.

Our studies on the *trpE26* and *trpE30* strains have demonstrated that in *B. subtilis* a single translocation may cause a series of other chromosomal rearrangements (duplications, inversions and deletions) when strains carrying it are crossed to the parental 168-type strain. The rearrangements involve extensive regions of the bacterial genome. It is probable that all rearrangements inducible in this system have not as yet been discovered. Those known so far do not seem to be deleterious to the cell. They ultimately result either in stable modifications of the genetic maps or in chromosome instability which re-establishes the genetic structure of the parental strains. The situation resembles in many respects that reported recently for *E. coli* (Hill & Harnish, 1981). Nevertheless in this case the chromosome area concerned is much smaller than those in *B. subtilis*.

Induction of chromosomal rearrangements is one of the processes by which evolution works. It will be interesting to use those induced by the *B. subtilis* systems we have described in conjunction with other procedures (for instance insertion of foreign genetic material into the chromosome via plasmids or phages) in order to promote evolution in the laboratory. Such studies may lead to (a) the creation of new *Bacillus* species, (b) increase in the synthesis of enzymes or of useful compounds in strains carrying stable duplications or triplications, and (c) gaining some insight into the mechanisms which ensure the perennality of a specific chromosome map configuration for each bacterial species.

We thank Dr S. A. Zahler for the gift of strain CU156. This work was supported by contract no. 225-76-1-810-F with the Commission of European Communities.

REFERENCES

- ANAGNOSTOPOULOS, C. (1977). Genetic analysis of *Bacillus subtilis* strains carrying chromosomal rearrangements. In *Modern Trends in Bacterial Transformation and Transfection*, pp. 211–230. Edited by A. Portolés, R. López & M. Espinosa. Amsterdam: Elsevier/North-Holland Biomedical Press.
- ANAGNOSTOPOULOS, C. & SPIZIZEN, J. (1961). Requirements for transformation in *Bacillus subtilis*. *Journal of Bacteriology* **81**, 741–746.
- ANAGNOSTOPOULOS, C. & TROWSDALE, J. (1976). Production of merodiploid clones in *Bacillus subtilis* strains. In *Microbiology – 1976*, pp. 44–57. Edited by D. Schlessinger. Washington, D.C.: American Society for Microbiology.
- ANDERSON, R. P. & ROTH, J. R. (1977). Tandem genetic duplications in phage and bacteria. *Annual Review of Microbiology* **31**, 473–505.
- ANDERSON, R. P. & ROTH, J. (1981). Spontaneous tandem genetic duplications in *Salmonella typhimurium* arise by unequal recombination between rRNA (*rnn*) cistrons. *Proceedings of the National Academy of Sciences of the United States of America* **78**, 3113–3117.
- AUDIT, C. & ANAGNOSTOPOULOS, C. (1972). Production of stable and persistent unstable heterogenotes in a mutant of *Bacillus subtilis*. In *Spores V*, pp. 117–125. Edited by H. O. Halvorson, R. Hanson & L. L. Campbell. Washington, D.C.: American Society for Microbiology.
- AUDIT, C. & ANAGNOSTOPOULOS, C. (1973). Genetic studies relating to the production of transformed clones diploid in the tryptophan region of the *Bacillus subtilis* genome. *Journal of Bacteriology* **114**, 18–27.
- AUDIT, C. & ANAGNOSTOPOULOS, C. (1975). Studies on the size of the diploid region in *Bacillus subtilis* merozygotes from strains carrying the *trpE26* mutation. *Molecular and General Genetics* **137**, 337–351.
- FINK, P. S., KORMAN, R. Z., ODEBRALSKI, J. M. & ZAHLER, S. A. (1981). *Bacillus subtilis* bacteriophage SP β cl is a deletion mutant of SP β . *Molecular and General Genetics* **182**, 514–515.
- HENNER, D. J. & HOCH, J. A. (1980). The *Bacillus subtilis* chromosome. *Microbiological Reviews* **44**, 57–82.
- HILL, C. W. & HARNISH, B. W. (1981). Inversions

- between ribosomal RNA genes of *Escherichia coli*. *Proceedings of the National Academy of Sciences of the United States of America* **78**, 7069–7072.
- HILL, C. W., GRAFSTROM, R. H. & HILLMAN, B. S. (1977*a*). Chromosomal rearrangements resulting from recombination between ribosomal RNA genes. In *DNA Insertion Elements, Plasmids and Episomes*, pp. 497–505. Edited by A. I. Bukhari, J. A. Shapiro & S. L. Adhya. New York: Cold Spring Harbor Laboratory.
- HILL, C. W., GRAFSTROM, R. H., HARNISH, B. W. & HILLMAN, B. S. (1977*b*). Tandem duplications resulting from recombination between ribosomal RNA genes in *Escherichia coli*. *Journal of Molecular Biology* **116**, 407–428.
- JAMET, C. & ANAGNOSTOPOULOS, C. (1969). Etude d'une mutation très faiblement transformable au locus de la thréonine désaminase de *Bacillus subtilis*. *Molecular and General Genetics* **105**, 225–242.
- LIPSKY, R. H., ROSENTHAL, R. & ZAHLER, S. A. (1981). Defective specialized transducing phages of *Bacillus subtilis* phage SP β that carry the *sup-3* or *sup-44* gene. *Journal of Bacteriology* **148**, 1012–1015.
- RILEY, M. & ANILIONIS, A. (1978). Evolution of the bacterial genome. *Annual Review of Microbiology* **32**, 519–560.
- ROSENTHAL, R., TOYE, P. A., KORMAN, R. Z. & ZAHLER, S. A. (1979). The prophage of SP β c2dcitK₁, a defective specialized transducing phage of *Bacillus subtilis*. *Genetics* **92**, 721–739.
- SCHNEIDER, A. M. & ANAGNOSTOPOULOS, C. (1981). Linkage map and properties of a *Bacillus subtilis* strain carrying a non-tandem duplication of the *purB*–*trc* region of the chromosome. *Journal of General Microbiology* **125**, 241–256.
- SCHNEIDER, A. M., GAISNE, M. & ANAGNOSTOPOULOS, C. (1982). Genetic structure and internal rearrangements of stable merodiploids from *Bacillus subtilis* strains carrying the *trpE26* mutation. *Genetics* **101**, 189–210.
- TROWSDALE, J. & ANAGNOSTOPOULOS, C. (1975). Evidence for the translocation of a chromosome segment in *Bacillus subtilis* strains carrying the *trpE26* mutation. *Journal of Bacteriology* **122**, 886–898.
- TROWSDALE, J. & ANAGNOSTOPOULOS, C. (1976). Differences in the genetic structure of *Bacillus subtilis* strains carrying the *trpE26* mutation and strain 168. *Journal of Bacteriology* **126**, 609–618.
- WARNER, F. D., KITOS, G. A., ROMANO, M. P. & HEMPHILL, H. E. (1977). Characterization of SP β : a temperate bacteriophage from *Bacillus subtilis* 168M. *Canadian Journal of Microbiology* **23**, 45–51.
- ZAHLER, S. A., KORMAN, R. Z., ROSENTHAL, R. & HEMPHILL, H. E. (1977). *Bacillus subtilis* bacteriophage SP β : localization of the prophage attachment site, and specialized transduction. *Journal of Bacteriology* **129**, 556–558.
- ZAHLER, S. A., KORMAN, R. Z., ODEBRALSKI, J. M., FINK, P. S., MACKEY, C. J., POUTRE, C. G., LIPSKY, R. & YOUNGMAN, P. J. (1982). Genetic manipulations with phage SP β . In *Molecular Cloning and Gene Regulation in Bacilli*, pp. 41–50. Edited by A. T. Ganesan, S. Chang & J. A. Hoch. New York: Academic Press.