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Mechanism of integrating foreign DNA during transformation of *Bacillus subtilis*

(heterospecific gene expression/recombinant molecules/plasmids/site-specific endonucleases/thymidylate synthetase)

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ABSTRACT **Genes encoding thymidylate synthetase from *Bacillus subtilis* bacteriophages were cloned in *Escherichia coli*. Chimeric plasmids pCD1 and pCD3 were constructed from site-specific endonuclease digests of bacteriophage ϕ3T DNA cloned in pMB9 in *E. coli*. Similar cloning techniques with bacteriophage β22 DNA yielded chimeric plasmids pCD4, pCD5, and pCD6. Endonuclease digests of DNA from pCD1 and pCD3 propagated in *E. coli* or from DNA isolated from bacteriophage ϕ3T propagated in *B. subtilis* transformed *B. subtilis* from Thy$^-$ to Thy$^+$. Intact DNA from bacteriophage β22, endonuclease digests of β22 DNA, and a chimeric plasmid (pCD5) composed only of the *thy*β22 gene and pMB9 did not transform *B. subtilis* from Thy$^-$ to Thy$^+$ even though pCD5 could transform Thy$^-$ *E. coli* to Thy$^+$. However, if the *thy*β22 fragment from pCD5 was introduced into another chimeric plasmid, pCD2, that contains a region of homology to the chromosome of *B. subtilis* in addition to pMB9, transformation of Thy$^-$ clones of *B. subtilis* was possible. Furthermore, Southern hybridization analyses of the digests of chromosomal DNA from the Thy$^+$ transformants established that the entire chimeric plasmid was incorporated into the chomosome of *B. subtilis*. Treatment of these plasmids with site-specific endonucleases abolished transformation. These results indicated that the entire chimeric plasmid can be incorporated into the chromosome of *B. subtilis* by a Campbell-like model. Therefore, an additional mechanism for transformation exists whereby plasmids can be integrated if sufficient chromosomal homology is maintained.**

Genetic studies have clearly established that the nucleotide homology in the region of recombination is the dominant factor regulating transformation by heterologous DNA. Accordingly, it is predicted that the introduction of regions of homology into plasmids, temperate bacteriophages, or chromosomes would foster transformation by these sources of foreign genes. Our previous studies with pCD1, a chimeric plasmid composed of pMB9 and the gene encoding thymidylate synthetase from bacteriophage ϕ3T, demonstrated that transformation of *Bacillus subtilis* by plasmids propagated in *Escherichia coli* was highly efficient (1). Transformation studies with pCD1 and other hybrid plasmids harboring regions of the ϕ3T genome suggested to us that such plasmids would not be ideal vectors for introducing foreign genes into *B. subtilis* for two reasons. First, Ehrlich *et al.* (2) demonstrated that the *thy*P3 region, but not the vector pSC101, was integrated into the chromosome of *B. subtilis*. Second, the gene encoding thymidylate synthetase in bacteriophage ϕ3T (*thy*P3) is similar to the chromosomal gene encoding thymidylate synthetase (*thy*A) in *B. subtilis* as evidenced by analysis of purified thymidylate synthetase* and DNA hybridization using the Southern technique (E. M. Rubin, personal communication). Third, the gene encoding thymidylate synthetase in pCD1 was integrated near the *thy*A locus in the chromosome of *B. subtilis* and not at the attachment site of bacteriophage ϕ3T (unpublished data).

We reasoned that, by using a nonhomologous *thy* gene cloned in a plasmid, we could select thymine prototrophs that were the result of transformation by foreign DNA either integrated into the chromosome or maintained as an autonomously replicating plasmid. In addition, foreign genes might be more readily incorporated if the vector contained a short region of homology with the chromosome of *B. subtilis*. This was accomplished by removing the segment encoding thymidylate synthetase (*thy*P3) from pCD1, leaving a new plasmid pCD2 that still retained a region of homology with the chromosome of *B. subtilis* but did not have any known genetic activity. Using this vector we have cloned the region encoding thymidylate synthetase from a virulent *B. subtilis* bacteriophage (β22) in *E. coli*. This gene, which we will designate as *thy*β22, unlike *thy*P3 from bacteriophage ϕ3T, does not have homology with the chromosome of *B. subtilis*. The data to be presented establish that the entire chimeric plasmid including the foreign gene, *thy*β22, and the plasmid pMB9 is incorporated within the chromosome by a mechanism of recombination that to our knowledge has heretofore been unreported in *B. subtilis*.

MATERIALS AND METHODS

Bacterial Strains and Bacteriophage. Thy$^-$ derivatives of *B.* subtilis 168 (RUB830) and *E. coli* C600 (RUE 1) were used in the study (1). Bacteriophage β22 was propagated in M medium as described by Yehle and Doi (3).

Genetic Analysis. The methods for the development of competence, transformation, and analysis of recombinants of *B. subtilis* were similar to those described previously (4). Most plasmids were isolated (5) and constructed (1) as described and subsequently introduced into *E. coli* by DNA-mediated transformation (6). In all instances the selected gene was *thy*β22, cloned from a *Bgl* II digest of DNA from bacteriophage β22. The combination of *Bgl* II and *Bam*HI is particularly useful in producing *in vitro* gene fusions as originally suggested by R. J. Roberts (7). Because *Bgl* II recognizes the site 5′-A$^{\downarrow}$GATCT (7, 8) and *Bam*HI, the site 5′G$^{\downarrow}$GATCC (9), a common internal sequence is generated, GATC. Thus, a new site, GGATCT, can be formed in the presence of ligase and either one of the enzymes and is now resistant to both endonucleases. This procedure provides a powerful selection for recombinant molecules *in vitro* and precludes the generation of the original parental molecules during ligation. The experiments were performed in accordance with the *NIH Guidelines for Recombinant DNA Research* at the P2 level of containment using an EK1 vector.

Physical Analysis. *Eco*RI (10), *Bam*HI (11), and *Bgl* II were purified by established procedures. Plasmids were treated with

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Abbreviation: Md, megadalton.

*Williams, M. T. & Young, F. E. (1977) *Abstracts, Annual Meeting, American Society of Microbiology*, p. 325.

these site-specific endonucleases and analyzed by ethidium bromide/agarose gel electrophoresis with adenovirus-2 DNA as an internal standard (1). Hybridization between plasmid probes prepared by nick translation (12) and site-specific endonuclease digestions of the chromosomal, bacteriophage, and plasmid DNAs were performed by a modification (13) of Southern's original technique (14). DNA polymerase I was purchased from Boehringer Mannheim (lot 1037120) and was found to have sufficient DNase activity to prime the nick-translation reaction. [α-^{32}P]dCTP (250 Ci/mmol) was purchased from Amersham Corp.

RESULTS

Development of Recombinant Plasmids. A summary of the physical maps of the plasmids used is shown in Fig. 1. Essentially, the *thy*P3 gene was excised from pCD1 by treatment with *Bgl* II and introduced into the *Bam*HI site of pMB9 by a *Bam/Bgl* fusion. *E. coli* strain RUE 1 was transformed for Thy$^+$ with this DNA. One transformant contained the plasmid designated pCD3. In other experiments the plasmid pCD1 was treated with *Bgl* II and ligase prior to transformation of RUE 1 to Tet^R. One Tet^R Thy$^-$ transformant contained the plasmid pCD2 (1). These results demonstrated that the gene *thy*P3 resides on the 1.5-megadalton (Md) fragment that is demarcated by the two *Bgl* II sites in pCD1. In other experiments, the 0.51-Md fragment of DNA from pCD2, derived from bacteriophage ϕ3T, was labeled by nick translation. Hybridization by the Southern technique established that the 0.51-Md segment has homology with a discrete *Eco*RI-generated fragment from the chromosome of *B. subtilis* (data not shown).

The presence of a gene encoding thymidylate synthetase in bacteriophage ϕ3T was readily apparent because lysogeny of Thy$^-$ mutants of *B. subtilis* by bacteriophage ϕ3T converted the lysogens to Thy$^+$ (15). Reasoning by analogy to the presence of genes regulating nucleotide metabolism in bacteriophage T4 (16), we designed an experiment to detect the presence of a gene encoding thymidylate synthetase in the virulent bacteriophage β22. DNA from bacteriophage β22 was cleaved into more than 50 fragments by *Bgl* II and was mixed with pCD2 that had been cleaved with *Bgl* II. After ligation and transformation of *E. coli* strain RUE 1, Thy$^+$ Tet^R transformants were selected. The plasmid in one of these was designated pCD4. The gene encoding thymidylate synthetase from bacteriophage β22 (*thy*β22) was found to reside on the 5.4-Md fragment in pCD4. This segment was subsequently excised with *Bgl* II and introduced into the *Bam*HI site of pMB9 and pCD2 by *Bam/Bgl* fusions, generating the plasmids pCD5 and pCD6, respectively (Fig. 1). The gel patterns used to develop the physical maps are shown in Fig. 2. The following points are critical for the analysis of subsequent experiments. First, pCD4 has two regions of ϕ3T DNA, 0.04 and 0.47 Md. In pCD6 these are joined to form a single 0.51-Md segment. Second, the plasmid pCD5 contains no DNA from bacteriophage ϕ3T because the β22 fragment was introduced directly into pMB9. Third, the *Eco*RI digests of pCD4, pCD5, and pCD6 will contain only one constant fragment (2.8 Md) because the construction of the plasmids results in altered arrangements in the other segments. Fourth, in pCD5 and pCD6 the 5.4-Md fragment was inserted in different directions, suggesting that the promoter for thymidylate synthetase is not on pMB9 or ϕ3T DNA but instead is probably in the fragment of β22 DNA. Fifth, there is one site in pCD4 (*Bam*HI) and in pCD6 (*Bgl* II) that can be used to cleave the plasmids into linear molecules without cleavage of the 5.4-Md segment of bacteriophage β22 DNA.

Thy$^+$ Transforming Activity of Plasmid, Bacteriophage, and Chromosomal DNA. The Thy$^+$ transforming activity of DNA was examined by incubating competent cells of *B. subtilis* strain RUB830 (*thy*A, *thy*B) with native DNA or DNA that was treated with site-specific endonucleases. As shown in Table 1,

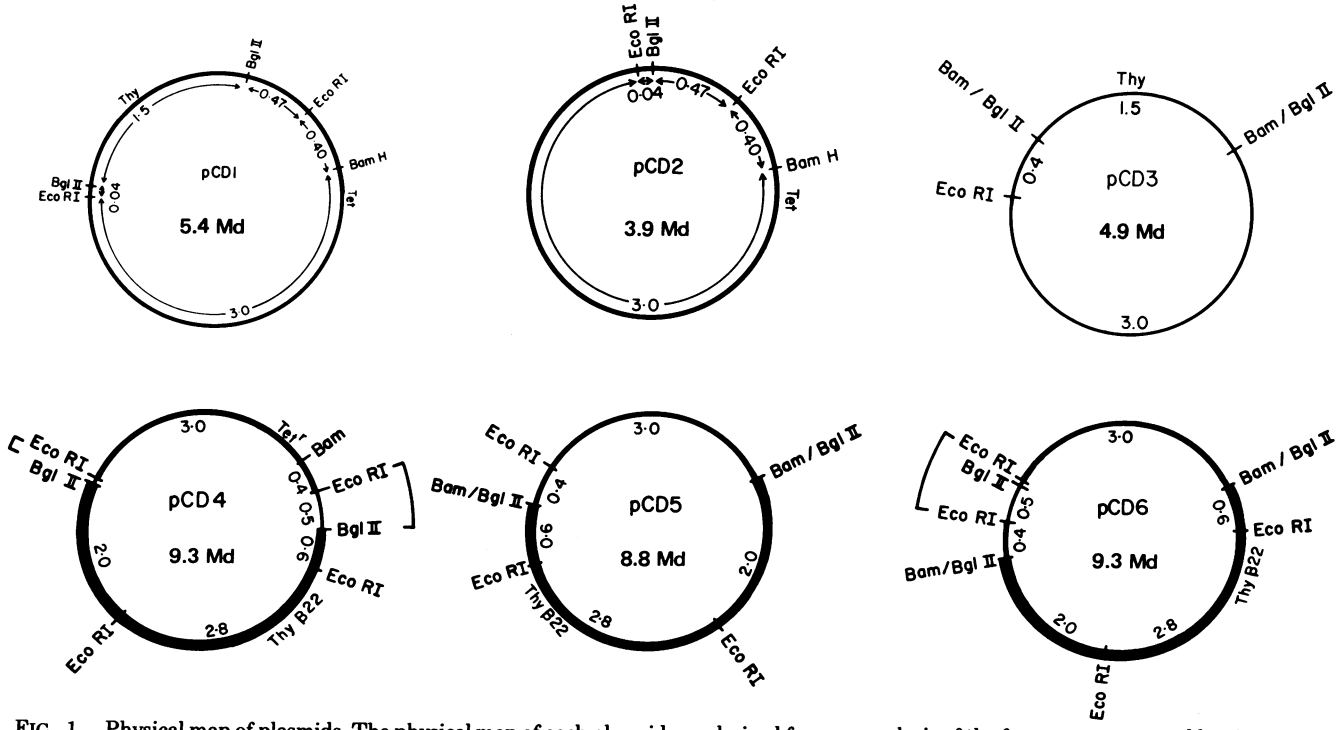

FIG. 1. Physical map of plasmids. The physical map of each plasmid was derived from an analysis of the fragments generated by site-specific endonucleases and shown for pCD4, pCD5, and pCD6 in Fig. 2. The location of cleavage by each endonuclease and the molecular masses (Md, megadaltons) of the fragment are included. For pCD4, pCD5, and pCD6, the fine lines defined by brackets denote the DNA segment(s) from bacteriophage ϕ3T, the moderately heavy line shows the DNA from pMB9, and the heavy line shows the DNA from bacteriophage β22.

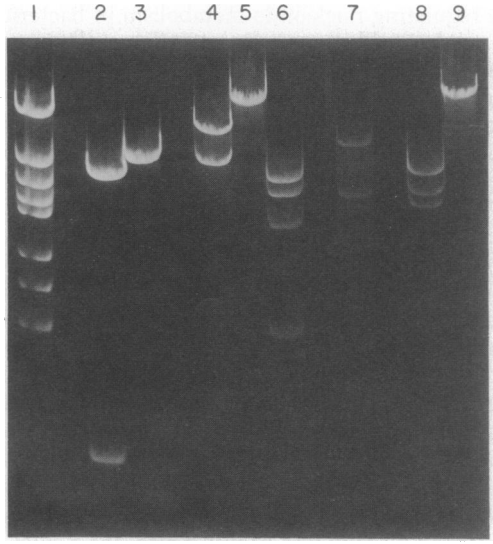

FIG. 2. Analysis of chimeric plasmids. Plasmids pCD2, pCD4, pCD5, and pCD6 were digested with *Eco*RI, *Bam*HI, and *Bgl* II and analyzed by agarose gel electrophoresis. Lanes: 1, *Eco*RI/*Bam*HI digest of adenovirus-2; 2, *Eco*RI digest of pCD2; 3, *Bgl* II digest of pCD2; 4, *Bgl* II digest of pCD4; 5, *Bam*HI digest of pCD4; 6, *Eco*RI digest of pCD4; 7, *Eco*RI digest of pCD5; 8, *Eco*RI digest of pCD6; 9, *Bgl* II digest of pCD6. The molecular masses of the adenovirus-2 fragments are 6.87, 3.44, 2.98, 2.52, 2.29, 1.83, 1.37, and 1.14 Md (Carel Mulder, personal communication).

we obtained 120,000 transformants per 10^8 recipient cells when 1 μg of wild-type *B. subtilis* 168 was used as the donor DNA. When pCD1 was used as the donor DNA, approximately 100,000 transformants per 10^8 recipient cells were obtained. No Tet^R colonies were detected in any of the Thy^+ clones obtained when any of the pCD plasmids was used to transform *B. subtilis* from Thy^- to Thy^+. Treatment of pCD1 with *Bam*HI (which linearizes the plasmid) or *Bgl* II (which excises the 1.5-Md fragment) resulted in 90,000 or 2000 transformants per 10^8 cells. The greater reduction of transformation after treatment with *Bgl* II is probably related to inefficient uptake of the smaller fragment by competent cells and is consistent with prevous results obtained with digestion of bacteriophage φ3T by *Bgl* II (17).

No Thy^+ transformants were obtained with pCD2, demonstrating the lack of the Thy^+ gene in this plasmid. On the other hand, 30,000 transformants were obtained with pCD3 when the DNA was introduced in either the circular or the linear form. Neither intact nor endonuclease-digested bacteriophage β22 DNA was able to transform *B. subtilis* from Thy^- to Thy^+. However, when Thy^- competent cells were incubated with closed circular DNA from pCD4 and pCD6, 92 and 172 transformants per 10^8 recipient cells were observed, respectively. When these plasmids were linearized with *Bam*HI or *Bgl* II, no transformants were identified. Furthermore, if the plasmids did not contain regions of bacteriophage φ3T DNA, as in the case of pCD5, no transformants were produced in *B. subtilis*.

Therefore, a small region of chromosomal homology introduced by bacteriophage φ3T DNA was required for the transformation of *B. subtilis* by plasmids carrying the *thyβ22* gene from bacteriophage β22. In other studies we examined the transformation of strain RUB830 with DNA extracted from Thy^+ transformants obtained by transformation of strain RUB830 with pCD4 DNA. The frequency of transformation was similar to that obtained initially with pCD4 or pCD6 DNA isolated directly from *E. coli* (Table 1).

Fate of Plasmids in Thy^+ Transformants. Three lines of evidence establish that the plasmids are not maintained as extrachromosomal elements in *B. subtilis* transformed by pCD4 and pCD6. First, no circular covalently closed DNA was found in lysates of transformed cells by techniques that have proven successful in identifying plasmids in *B. subtilis* (18). Second, we isolated DNA from transformants and analyzed the preparation by using a nick-translated pMB9 probe with the Southern hybridization technique. As shown in Fig. 3, the probe bound to the high molecular weight chromosomal DNA. No DNA was identified in and no binding was seen to the region of the gel corresponding to a 9.3-Md plasmid (Fig. 3, lanes C and c). After treatment of the same DNA from the transformant with *Eco*RI, the probe hybridized to a segment of DNA that migrated to the position in the gel (lane d) corresponding to linear pMB9 (lane e). Third, pCD5, a plasmid that contains all of the sequences of pCD4 and pCD6 except the 0.51-Md φ3T segment, did not transform *B. subtilis* to Thy^+.

Table 1. Thy^+ transforming activity of various DNA preparations

DNA	No. of transformants after treatment with			
	No enzyme	*Bam*HI	*Bgl* II	*Eco*RI
Wild-type chromosomal	120,000	ND	ND	ND
pCD1	100,000	90,000	2000	ND
pCD2	0	0	0	0
pCD3	30,000	ND	ND	30,000
pCD4	92	0	0	0
Intergenote (pCD4/RUB830)	250	ND	ND	ND
pCD6	172	ND	0	ND
pCD5	0	ND	ND	ND
β22	0	ND	0	0

DNA from various sources was used to transform *B. subtilis* 168 strain RUB830. Transformants are expressed per 10^8 cells. ND denotes not determined.

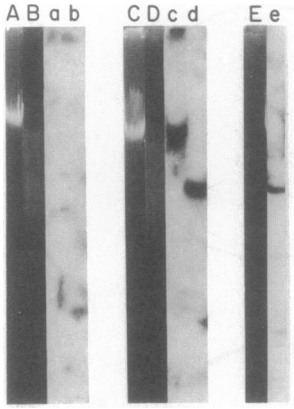

FIG. 3. Hybridization of pMB9 to chromosomal DNA. DNAs were electrophoresed on agarose gels, denatured *in situ*, eluted, and transferred to nitrocellulose paper. The nitrocellulose was bathed for 12 hr at 68° in 0.9 M NaCl/0.09 sodium citrate containing 1 μg of pMB9 DNA that was labeled by nick translation with [α-^{32}P]dCTP. After being washed at 68° in 0.3 M NaCl/0.03 sodium citrate/0.5% sodium dodecyl sulfate, the paper was dried and mounted for autoradiography. (A–E) Ethidium bromide/agarose gels. Lanes: A, RUB830 DNA (1 μg); B, RUB830 DNA (1 μg) cleaved with *Eco*RI; C, DNA (1 μg) from RUB830 transformed by pCD4; D, DNA (1 μg) from RUB830 transformed by pCD4 that had been cleaved with *Eco*RI; E, linear pMB9 DNA (50 pg). (a–e) The autoradiograms obtained after hybridization of radioactive pMB9 with the digests shown in A–E.

Although extrachromosomal plasmids could not be detected in transformants of *B. subtilis*, the entire plasmid could be identified after *Eco*RI digestion of the chromosome of the transformant. In these studies we digested bacteriophage β22 DNA with *Bgl* II and *Eco*RI and the chromosomal DNA from the transformants, with *Eco*RI. After ethidium bromide/agarose gel electrophoresis, hybridization was determined by the Southern technique using nick-translated pCD5. Homology between the gene encoding thymidylate synthetase from bacteriophage β22 and pCD5 was established by the hybridization studies using an *Eco*RI digest of DNA from β22 (lanes A and a of Fig. 4) or a *Bgl* II digest of DNA from β22 (lanes B and b). For reference purposes, a *Bgl* II digest of pCD4 (lane G) is shown on the same figure. In lane G, the band B2 is the cloning vector, pCD2, and B1 is the cloned fragment of β22 DNA. Note that the hybridization probe, pCD5, bound to a single band in lane b and that this band comigrated with fragment B1, indicating that only a single *Bgl* II fragment carrying *thy*β22 was cloned. Furthermore, it was cloned without deletions. Lane a shows binding to three *Eco*RI fragments of β22 (bands 2, 5, and 8), confirming the existence of two *Eco*RI sites in the cloned *Bgl* II fragment.

Fig. 4 also summarizes our studies on hybridization of *Eco*RI digests of chromosomal DNA from *B. subtilis* RUB830 (lane c) and Thy$^+$ clones of strain RUB830 that had been transformed with plasmids pCD4 and pCD6 (lanes d and e, respectively). It is important to note that there was no hybridization between an *Eco*RI digest of the chromosome of *B. subtilis* strain RUB830 and the radioactive plasmid pCD5. This control, shown in lanes C and c, established that there was no homology between the chromosome, the vector pMB9, or the gene encoding thymidylate synthetase from bacteriophage β22. In lane d, four bands were observed in an *Eco*RI digest of chromosomal DNA (bands 4, 5, 7, and 9). Comparison of lane d with an *Eco*RI digest of pCD4 (lane F) shows that fragment E1 comigrated with band 4, fragment E2 with band 5, and fragment E3 with band 7. However, fragment E4 migrated more slowly than band 9, indicating that the integration event occurred within this 1.1-Md *Eco*RI fragment of pCD4 containing the 0.5-Md fragment of φ3T DNA. In lane e, three radioactive bands are seen in the *Eco*RI digest of the DNA from a pCD6 transformant. These bands (bands 3, 5, and 6) correspond exactly to the larger *Eco*RI fragments of pCD6 (see Figs. 1 and 3). There was no hybridization to the fastest migrating band because the probe, pCD5, lacks this sequence.

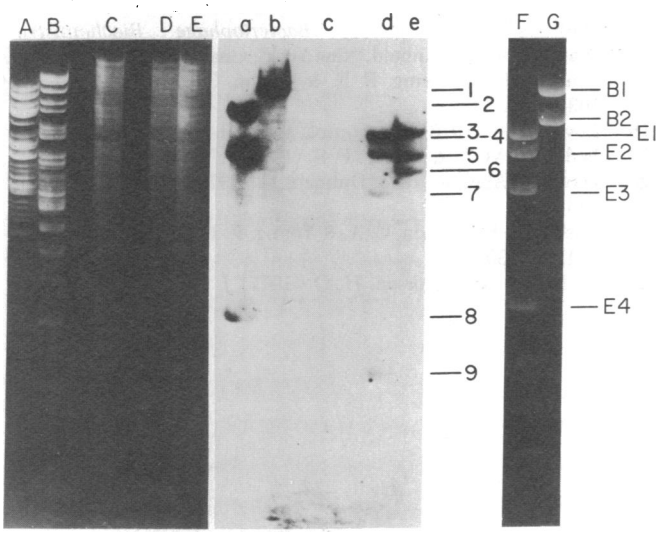

FIG. 4. Hybridization of pCD5 to bacteriophage and chromosomal DNA. Each DNA (1 μg) was electrophoresed on agarose gels and hybridized to pCD5 DNA that had been prepared by nick-translation as described in Fig. 3. The autoradiogram (lanes a–e) is shown between photographs of the ethidium bromide/agarose gels. Lanes: A, *Eco*RI digest of β22 DNA; B, *Bgl* II digest of β22 DNA; C, *Eco*RI digest of DNA from *B. subtilis* strain RUB830; D, *Eco*RI digest of DNA from *B. subtilis* strain RUB830 that was transformed with pCD4 to Thy$^+$; E, *Eco*RI digest of DNA from *B. subtilis* strain RUB830 that was transformed with pCD6 DNA to Thy$^+$; F, *Eco*RI digest of pCD4; and G, *Bgl* II digest of pCD4. Lanes a–e display the autoradiograms obtained by hybridization of radioactive pCD5 with the respective digests shown in lanes A–E. These bands are numbered 1–9. Bands E1–E4 and B1 and B2 denote the *Eco*RI and *Bgl* II fragments of pCD4, respectively, that are shown in lanes F and G.

Because pCD6 is an *in vitro* rearrangement of pCD4 produced by a *Bam/Bgl* fusion, the molecular weights of the fragments were different and fewer fragments were observed in digests of clones transformed by pCD6 than by pCD4. Nevertheless, analysis of the known fragments of pCD4 and pCD6 in digests of the transformants by using the probe pCD5 established that all of the known *Eco*RI fragments of the plasmids can be accounted for. Furthermore, as discussed below, these patterns are consistent with the integration of the plasmid within the region of φ3T DNA.

DISCUSSION

To study the factors regulating the incorporation of foreign genes in *B. subtilis*, we have developed chimeric plasmids that can be amplified in *E. coli* and can be subsequently used to transform *B. subtilis*. Because thymidylate synthetase is not a highly regulated enzyme, it has been extremely useful in these studies. Hybridization analysis of the cloned fragments containing the genes encoding thymidylate synthetase from bacteriophage φ3T and β22 demonstrates that the former has major homology with the chromosomal gene in *B. subtilis* (*thy* A) whereas the latter has no significant homology. The data presented in this study establish: (*i*) the vector pMB9 has no homology with the chromosome of *B. subtilis*; (*ii*) chimeric plasmids composed only of pMB9 and the 5.4-Md fragment carrying *thy*β22 from bacteriophage β22 cannot transform *B. subtilis* but they can transform Thy$^-$ *E. coli* to Thy$^+$ and exist as a plasmid in this organism; (*iii*) chimeric plasmids containing small regions of bacteriophage φ3T DNA but none of the gene encoding thymidylate synthetase (*thy*P3) from bacteriophage φ3T transform both *B. subtilis* and *E. coli* from Thy$^-$ to Thy$^+$; (*iv*) in *E. coli* transformants, the entire plasmid is maintained as an extrachromosomal element whereas in *B. subtilis* the entire plasmid is incorporated into the chromosome.

At present we do not know the location of the *thy*β22 gene in the chromosome of the Thy$^+$ transformants of *B. subtilis*. Based on mapping studies with transformants obtained from intact bacteriophage φ3T DNA, *Bam*HI digests of bacteriophage φ3T DNA, and pCD1 (19),* it is likely that integration of pCD4 and pCD6 occurs near the chromosomal attachment site of bacteriophage φ3T or near the *thy*A gene. Because the isolated segment of bacteriophage φ3T DNA cloned in pCD1 integrates near *thy*A, we suspect that the 0.51-Md fragment of bacteriophage φ3T DNA in pCD4 and pCD6 would have homology with the chromosome of *B. subtilis* near *thy*A. On the other hand, all recombination may not require detectable chromosomal homology.

Circular DNA is necessary for transformation of *B. subtilis* by pCD4 and pCD6 (Table 1) but not by pCD1. This observation and the requirement for a short stretch of chromosomal sequence homology for the transforming activity of these plasmids suggest the following models. Integration of DNA may

occur by two pathways in *B. subtilis*. Usually, double-stranded DNA is converted first to double-stranded fragments, then to single-stranded fragments, and finally integrated as single-stranded regions into the chromosome of *B. subtilis* (20), presumably at gaps (21). Alternatively, circular double-stranded DNA can enter the cell and not be processed to single-stranded intermediates. This circular DNA then recombines by a reciprocal crossover event. This pathway is used infrequently and cannot be seen unless the more frequent classical single-stranded integration is precluded.

Direct hybridization of probes with digests of chromosomal DNA provides a powerful tool for evaluating this model of recombination. If the entire plasmid bearing several endonuclease sites is integrated into the chromosome at a specific site on the plasmid, then only one endonuclease-generated fragment will be altered with respect to mobility on the gels. All other fragments must remain the same. The altered fragment should now appear as two new bands on the autoradiographic pattern. The molecular weights of these bands will depend on the location of the endonuclease sites within the chromosome. The data presented in Fig. 4 are consistent with these predictions. Note that the bands bordered by *Eco*RI sites in the plasmid are identical to those in the chromosome of a clone transformed with pCD4 or pCD6, suggesting that the recombination occurred within the small *Eco*RI fragment from bacteriophage ϕ3T which also shares homology with the chromosome. The altered migration of band 9 is expected because integration occurred within this region. Due to the configuration of the transforming plasmid and the radioactive probe, it is not possible to detect the other end of the chromosome that participated in the recombinational event. The increased migration of the band 9 relative to band E4 suggests that the recombinational event occurred near an *Eco*RI site on the chromosome.

At present we have only one additional experiment that can be used to evaluate this model. We constructed a plasmid, pCD9, that was identical to pCD6 except that the 0.51-Md fragment of bacteriophage ϕ3T was replaced by insertion of a 1-Md *Eco*RI fragment of the chromosome of *B. subtilis* that complements the *leu*B locus in *E. coli* HB101. This fragment is presumably identical to that cloned by other investigators (2, 22). Thy$^+$ transformants occurred at a higher frequency than with pCD4 or pCD6, suggesting that the frequency of recombination may be related to the extent of chromosomal homology.

Based on the known requirements for transformation in *B. subtilis*, an additional prediction can be made. If an organism contains a foreign plasmid such as pMB9 that was fused into the chromosome during recombination, it should be possible to recombine other pMB9 chimeric plasmids at this site.

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1. Duncan, C. H., Wilson, G. A. & Young, F. E. (1977) *Gene* **1**, 153–167.
2. Ehrlich, S. D., Bursztyn-Pettegrew, H., Stroynowski, I. & Lederberg, J. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 4145–4149.
3. Yehle, C. O. & Doi, R. H. (1967) *J. Virol.* **1**, 935–947.
4. Boylan, R. J., Mendelson, N. H., Brooks, D. & Young, F. E. (1972) *J. Bacteriol.* **110**, 281–290.
5. Clewell, D. B. & Helinski, D. R. (1969) *Proc. Natl. Acad. Sci. USA* **62**, 1159–1166.
6. Cohen, S. N., Chang, A. C. Y. & Hsu, L. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 2110–2114.
7. Roberts, R. J. (1977) in *Recombinant Molecules: Impact on Science and Society*, eds. Beers, R. F. & Bassett, E. G. (Raven, New York), pp. 21–32.
8. Pirrotta, V. (1976) *Nucleic Acids Res.* **3**, 1747–1760.
9. Roberts, R. J., Wilson, G. A. & Young, F. E. (1977) *Nature* **265**, 82–84.
10. Wilson, G. A., Williams, M. T., Baney, H. W. & Young, F. E. (1974) *J. Virol.* **14**, 1013–1016.
11. Wilson, G. A. & Young, F. E. (1975) *J. Mol. Biol.* **97**, 123–125.
12. Rigby, P. W., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113**, 237–251.
13. Botchan, M., Topp, W. & Sambrook, J. (1976) *Cell* **9**, 269–287.
14. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
15. Tucker, R. G. (1969) *J. Gen. Virol.* **4**, 489–504.
16. Mathews, C. K. (1971) *Bacteriophage Biochemistry* (Van Nostrand-Reinhold, New York), Chapter 6.
17. Graham, R. S., Young, F. E. & Wilson, G. A. (1977) *Gene* **1**, 169–180.
18. Lovett, P. S. (1973) *J. Bacteriol.* **115**, 291–298.
19. Williams, M. T. & Young, F. E. (1977) *J. Virol.* **21**, 522–529.
20. Davidoff-Abelson, R. & Dubnau, D. (1973) *J. Bacteriol.* **116**, 154–162.
21. Yasbin, R. E., Wilson, G. A. & Young, F. E. (1975) *J. Bacteriol.* **121**, 296–304.
22. Mahler, I. & Halvorson, H. O. (1977) *J. Bacteriol.* **131**, 374–377.