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Instability of rRNA Operons in *Bacillus subtilis*

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Many laboratory strains of *Bacillus subtilis* contain 9 rather than 10 rRNA operons due to deletions occurring within the *rrnJ-rrnW* or *rrnI-rrnH-rrnG* gene cluster. These operons are members of two sets of closely spaced clusters located in the *cysA-aroI* region. Analysis of rescued DNA from integrants with insertions into *rrnG* and *rrnH* indicated that these tandemly arranged operons allowed frequent deletions of an *rrn* operon equivalent. These events may arise spontaneously by intrachromosomal recombination or by simultaneous double crossovers with a multimeric integrative plasmid.

The endospore-forming bacterium *Bacillus subtilis* has 10 rRNA gene sets, of which 9 are clustered between the origin and 75 degrees on the genomic map (1, 8) (Fig. 1). In a separate study, we demonstrated by integrative mapping, Southern analysis, and transductional crosses two well-separated sets of closely spaced *rrn* clusters in the region between *cysA14* and *aroI906* (Fig. 1). The first two, *rrnJ* and *rrnW*, are assigned to *Bcl*I homologs of 4.9 and 5.8 kilobases (kb), respectively, are located between *cysA14* and *rpsE2*. The second set, *rrnI*, *rrnH* and *rrnG*, of which the latter two are assigned to *Bcl*I homologs of 5.5 and 4.8 kb, respectively, are located between *att*SPO2 and *glpT6* (E. D. Jarvis, R. L. Widom, G. LaFauci, Y. Setoguchi, I. R. Richter, and R. Rudner, manuscript in preparation) (Fig. 1). Southern analysis showed that in certain common laboratory strains of *B. subtilis*, the number of *Bcl*I *rrn* homologs is 9 instead of 10, revealing the loss of either the 4.8- or the 5.8-kb fragments assigned to *rrnG* and *rrnW*, respectively. Our earlier studies with strains of the first group (6) and similar analyses in another laboratory (10) revealed the loss of a ribosomal homolog in other restriction enzyme digests of genomic DNA. During transformation with integrable plasmids containing rDNA, several strains were created which resembled the naturally occurring deletion strains with respect to their *Bcl*I and *Eco*RI Southern patterns.

We report that rescue of DNA from the chromosomal region of *rrn* deletions indicates that the naturally occurring deletions arose by homologous recombination within the closely spaced operons *rrnJ-rrnW* and *rrnI-rrnH-rrnG*. Restriction analyses of rescued DNA from the region of the deletion provide additional evidence for the existence of *rrnI*, the third operon in the second cluster, whose location was predicted by the heteroduplex mapping of Chow and Davidson (2) but so far has had no plasmid insertions.

MATERIALS AND METHODS

Bacterial strains and plasmids. Some of the *B. subtilis* strains and plasmids (pGR102 and pWR103) used in this study were described previously (8). Integrable plasmid pGR110, which contains a 0.5-kb *Pst*I-*Sma*I 16S fragment, has been described (G. LaFauci, Ph.D. dissertation, City University of New York, 1987). Integrant strains 168T-72 and 168T-77 contain pWR103; BD170-20 contains pGR102; SB25-40 and BD170-12 contain pGR110. Plasmid pGS227 (11) containing two contiguous *Eco*RI fragments (1.9 and 1.1 kb) derived from the 5′ terminus of the *B. subtilis rrnB* operon was obtained from K. Bott. Rescued plasmids from strains SB25-40 and BD170-12 (see below) were transformed into *Escherichia coli* HB101, and clones were selected for their tetracycline resistance on LB plates containing 15 μg of tetracycline per ml.

DNA manipulation. DNA preparations were carried out as described previously (8). Chromosomal DNA was restricted for Southern hybridizations as described previously (8). For plasmid rescue, DNA from strains SB25-40 and BD170-12 was partially restricted with *Eco*RI (1 to 3 U/μg of DNA for 1.5 to 5 h at 37°C), self-ligated at low (2 μg/ml) DNA concentration with 0.5 Weiss Units of T4 DNA ligase (New England Biolabs, Beverly, Mass.) per ml, and incubated at 4°C for 18 h.

DNA labeling and Southern blotting. All procedures for labeling and blots were described previously (8).

RESULTS

Distribution of *rrn* homologs in natural deletion strains of *B. subtilis*. Southern analysis of *Bcl*I genomic digests probed with [^{32}P]rDNA (Fig. 2a) revealed a consistent strain heterogeneity. In general, *B. subtilis* strains are found to contain either 9 or 10 operons. The genomic assignment of the *Bcl*I fragments shown in Fig. 2a was established through integrative mapping and transductional crosses (Fig. 1) (8; Jarvis et al., manuscript in preparation). Strains like BD170 (*trpC2 thr-5*) and its derivatives showed nine *rrn* gene sets; they were missing the 4.8-kb *Bcl*I homolog associated with *rrnG* (8) (Fig. 2a). Strains like CU420 (*trpC2 leuB6 ilvC4*) were missing the 5.8-kb *Bcl*I homolog associated with *rrnW* (Fig. 2a).

After restriction of genomic DNAs with *Eco*RI and hybridization to radioactively labeled 23S sequences, 9 or 10 fragments were produced on Southern blots. A missing *Bcl*I homolog can be easily correlated with the absence of a unique *Eco*RI fragment derived from portions of a doublet with a short DNA spacer (Fig. 3c). Figure 2b illustrates 10 well-resolved *Eco*RI fragments, ranging from 2.3 to 9.3 kb, produced from the common *B. subtilis* strain 168T. Strains with deletions (BD170 or CU420) exhibited losses of the 2.9- or 3.9-kb *Eco*RI fragment, respectively (Fig. 2b and 3). Among many common laboratory strains of *B. subtilis* examined, only these two unique *Eco*RI and *Bcl*I homologs were shown to be missing.

The closely clustered operons *rrnJ-rrnW* and *rrnI-rrnH-*

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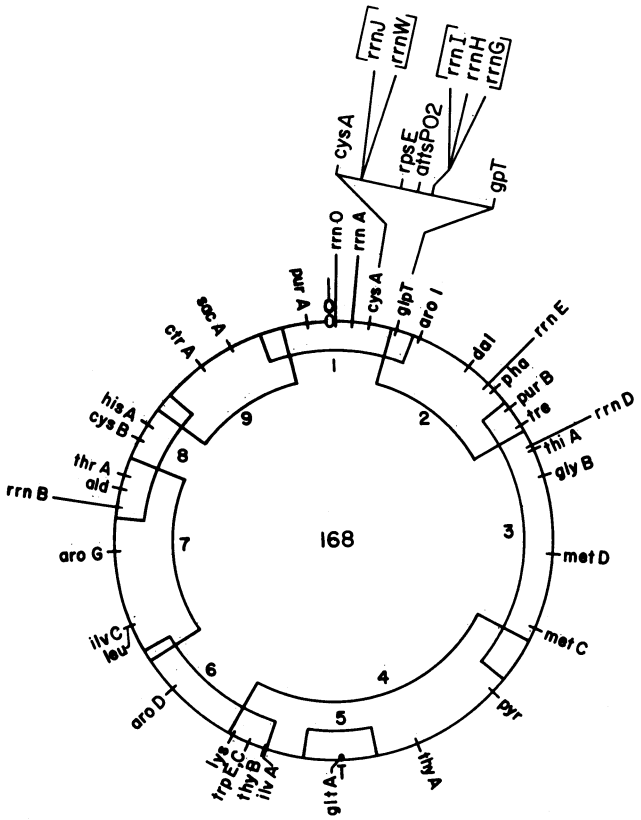

FIG. 1. The genetic map of *B. subtilis*, with the position of all 10 rRNA genes shown. The basic reference for the known loci is taken from the mapping kit strains of Dedonder et al. (3), which are designated 1 to 9. The transduction maps for the two clusters (*rrnJ-rrnW* and *rrnI-rrnH-rrnG*) will be reported separately (Jarvis et al., manuscript in preparation).

rrnG presented a problem in the *Bcl*I homolog assignments since they contain fragments of two neighboring *rrn* gene sets (Fig. 3c). A similar problem arises in assigning operon deletions. As will be shown below, the 4.8-kb *Bcl*I and the 2.9-kb *Eco*RI fragments represent rDNA containing portions of both *rrnH* and *rrnG* with the intergenic spacer between them (Fig. 3c). By our convention, when the above fragments are missing, we describe the strain as being deleted for *rrnG*, even though the intergenic spacer is also missing (Fig. 3c). The same convention applies for deletions in the second cluster of *rrnJ-rrnW* (Fig. 3c).

Distribution of *rrn* homologs in integrant strains with deletions induced by plasmid insertion. During plasmid transformation with recipients containing 10 *rrn* operons, several strains were created which resembled the naturally occurring deletion strains with respect to their *Bcl*I and *Eco*RI hybridization patterns. As discussed previously (8), in a normal Campbell-like integration event without a deletion, a single *rrn* operon is involved, leading to a shift in size of a single *Bcl*I homolog, with no change in the *Eco*RI pattern. The *Bcl*I homolog is increased in size due to the insertion of vector sequences. The *Eco*RI pattern is unchanged due to the presence of an *Eco*RI site downstream of the plasmid insertion and to the use of a 23S probe. Any normal integrants revealed *Eco*RI patterns indistinguishable from those of the parental strain 168T (Fig. 2b and data not shown).

Some integrant strains, however, showed two missing *Bcl*I homologs with the appearance of a single high-molecular-weight *Bcl*I fragment as well as the absence of a single *Eco*RI 23S homolog (Fig. 2a and b). Because these integrants displayed only a single high-molecular-weight *Bcl*I fragment, it became clear that only a single insertion had taken place, with a concomitant deletion of rDNA. As shown in Fig. 2a, integrant strain 168T-72 lost two *Bcl*I homologs, the 4.8- and 5.5-kb species associated with *rrnG* and *rrnH*, respectively, and exhibited the appearance of a single high-molecular-weight band of 11.3 kb. The loss of the two homologs was not associated with the simultaneous appearance of two high-molecular-weight bands (10.5 and 11.3 kb) which would correspond to separate integrations of plasmids into two homologs (Fig. 2a). Instead, the high-molecular-weight fragment of 11.3 kb in the *Bcl*I digestion represented a plasmid inserted into a 5.5-kb homolog (*rrnH*), yet the 4.8-kb homolog was also missing. The *Eco*RI pattern probed with 23S sequences for this strain was missing the 2.9-kb fragment. The missing *Eco*RI and *Bcl*I fragments were identical to the ones missing in strain BD170 by a natural deletion (Fig. 2a and b). The *Eco*RI pattern also showed the location of the plasmid-containing band which was 5.6 kb (pWR103), indicating that no alterations occurred to the vector upon integration.

Similarly, strain 168T-77 showed an insertion into the 4.9-kb *Bcl*I fragment (*rrnJ*) with the corresponding deletion

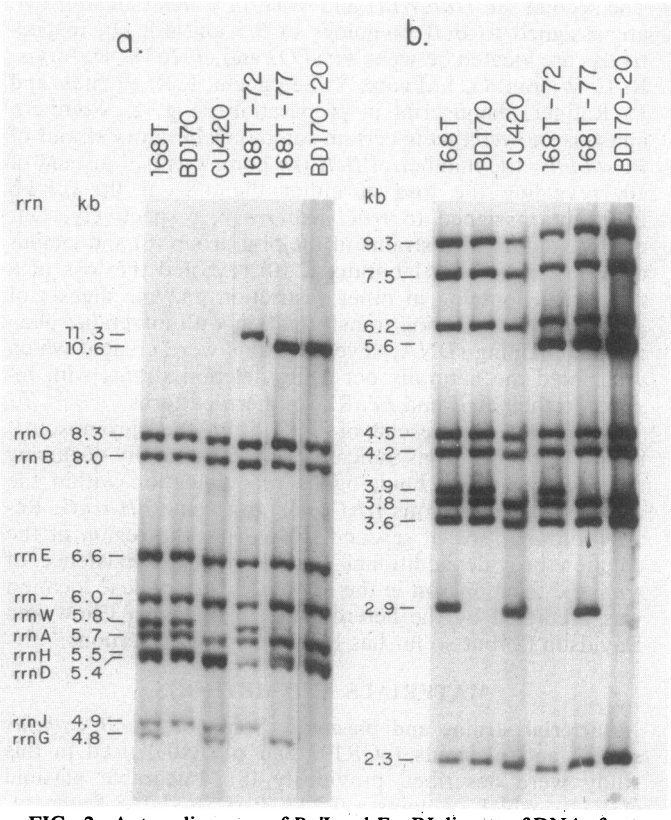

FIG. 2. Autoradiograms of *Bcl*I and *Eco*RI digests of DNAs from strains with naturally occurring *rrn* deletions or those with deletions induced by insertion of plasmids containing *rrn* sequences. (a) *Bcl*I digests electrophoresed on 0.75% agarose for 5 to 7 days at 15 to 20 mA and probed with a labeled *Eco*RI-*Pst*I 23S fragment from pYR104 (Jarvis et al., manuscript in preparation). (b) *Eco*RI digests electrophoresed on 0.75% agarose for 48 h at 15 to 20 mA and probed with a labeled *Eco*RI-*Hin*dIII 23S, 5S fragment from pYR104.

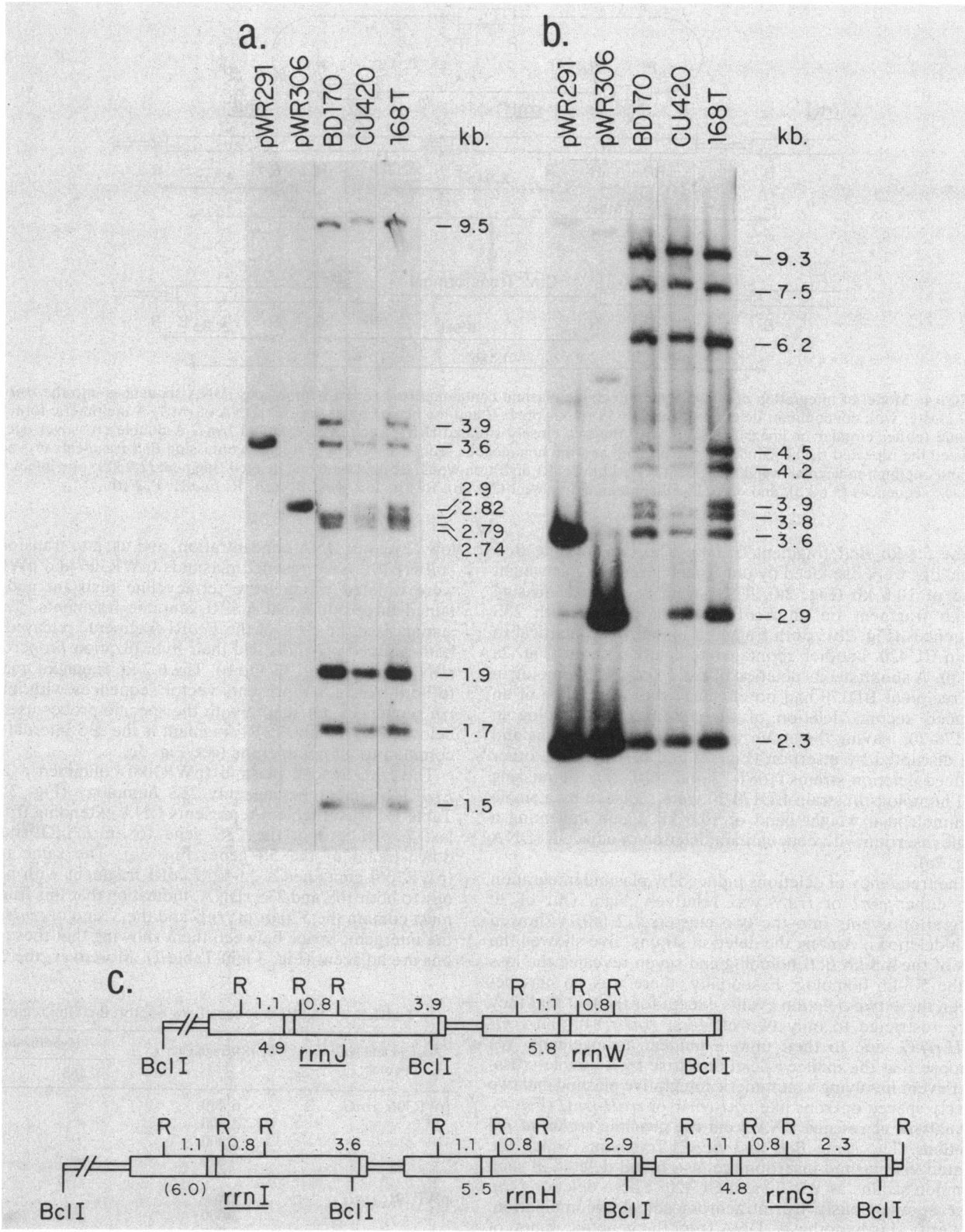

FIG. 3. Autoradiograms of *Eco*RI-digested chromosomal DNAs and rescued plasmids pWR306 and pWR291. The blots were probed as follows: (a) 1.9-kb *Eco*RI fragment from pGS227 (11) containing the 5′ end of the 16S gene; (b) 23S 1.2-kb *Eco*RI-*Pst*I fragment from pYR104. (c) Structures of the doublet *rrnJ-rrnW* located between *cysA14* and *rpsE2* and the triplet *rrnI-rrnH-rrnG* located between *att*SPO2 and *glpT6*. The *Bcl*I and *Eco*RI (R) fragment sizes shown are those identified by restriction analysis.

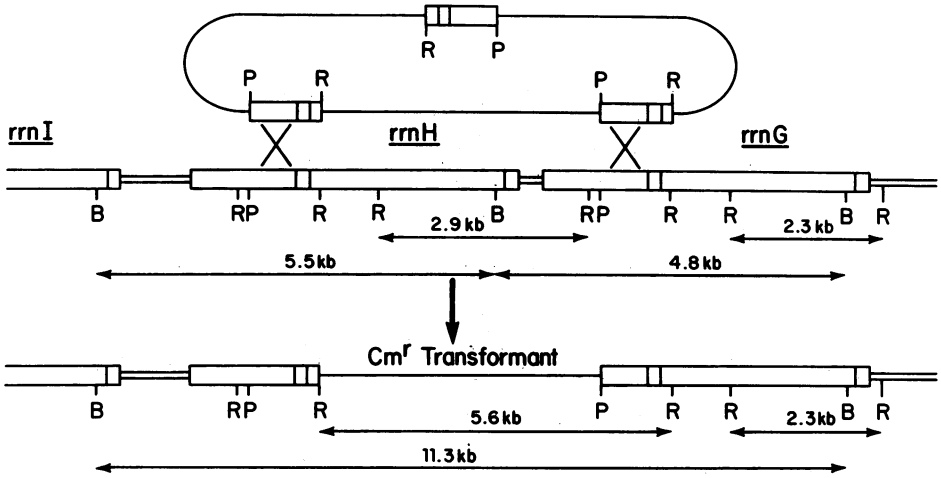

FIG. 4. Model of integration of a prototype integrable plasmid containing *PstI-EcoRI*-homologous rDNA sequences into the *rrnI-rrnH-rrnG* cluster with concomitant deletion of the 23S-5S rDNA of *rrnH* and the beginning of the 16S rDNA of *rrnG*. A multimeric form of the plasmid (either circular or linear) interacts with the two closely situated rRNA gene sets *rrnH* and *rrnG*. A double crossover occurring between the repeated *rrn* sequences of the plasmid and the homologous sequences would generate two missing *BclI* fragments (5.5 and 4.8 kb) and one high-molecular-weight fragment (11.3 kb). *EcoRI* digestion would demonstrate one deleted fragment (2.9 kb), one intact vector plus *rrn* sequences (5.6 kb), and one 23S-5S-containing fragment (2.3 kb). Restriction sites: B, *BclI*; R, *EcoRI*; P, *PstI*.

of the 5.8-kb *BclI* fragment (*rrnW*). The two missing *BclI* homologs were displaced by only one high-molecular-weight band of 10.6 kb (Fig. 2a). This strain revealed a missing 3.9-kb fragment on an *EcoRI* pattern probed with 23S sequences (Fig. 2b). Both fragment losses were identical to strain CU420, another spontaneous deletion strain (Fig. 2a and b). A spontaneous deletion of the 4.8-kb *BclI* homolog in the recipient BD170 had no effect on the occurrence of an induced second deletion of the 5.8-kb *BclI* homolog in BD170-20, leaving the strain with seven intact operons and one disrupted by insertion (Fig. 2a and b). As in the other induced deletion strains (168T-72 and 168T-77), the missing *BclI* homologs in strain BD170-20 were replaced by a single high-molecular-weight band of 10.6 kb, again indicating a single insertion with concomitant deletion of adjacent rDNA (Fig. 2a).

The frequency of deletions induced by plasmid integration into either *rrnJ* or *rrnH* was relatively high. Out of 30 integration events into the two clusters, 12 (40%) showed such deletions. Among the deletion strains, five showed the loss of the 4.8-kb *BclI* homolog and seven revealed the loss of the 5.8-kb homolog. Essentially, there was no bias between these two deletion events except for the fact that they were restricted to only two clusters, *rrnJ-rrnW* and *rrnI-rrnH-rrnG*, due to their unique tandem arrangement. We propose that the induced deletions arise by a double crossover event involving a multimeric integrative plasmid and two closely spaced operons like *rrnJ-rrnW* or *rrnH-rrnG* (Fig. 4).

Analyses of rescued DNA from the genomic region of *rrn* deletions. The same *BclI* and *EcoRI* fragments which are deleted via plasmid insertion are also found deleted in such common strains as BD170 and CU420. These deletions can arise spontaneously by intrachromosomal recombination. To verify this hypothesis, DNA from the genomic region of *rrnG* and *rrnH* was cloned and analyzed. Integrant strains with insertions of pGR110 into *rrnG* (SB25-40) and *rrnH* (BD170-12) were chosen for these analyses. The latter strain contains a natural deletion of the 4.8-kb *BclI* fragment from the *rrnI-rrnH-rrnG* cluster. DNA from these two integrant strains was partially restricted with *EcoRI*, self-ligated at low (2 μg/ml) DNA concentration, and used to transform *E. coli* HB101. Two rescued plasmids (pWR306 and pWR291) were isolated which were tetracycline resistant and contained three additional *EcoRI* genomic fragments. Table 1 summarizes the sizes of the *EcoRI* fragments released from both rescued plasmids and their hybridization properties to rDNA probes (Fig. 3a and b). The 6.2-kb fragment common to both plasmids represents vector sequences with internal *rrn* sequences not shared with the specific probes used (see below). The 0.8-kb *EcoRI* fragment is the 23S internal piece common to all *rrn* operons (see Fig. 3c).

The *rrnG* rescue plasmid (pWR306) contained a 2.3-kb *EcoRI* fragment having only 23S homology (Fig. 3b and Table 1). This fragment represents DNA extending from the last *EcoRI* site in the 23S gene to an *EcoRI* site just downstream of the 5S gene (Fig. 3c). The same rescue (pWR306) contained a 2.9-kb *EcoRI* fragment with homology to both 16S and 23S rDNA, indicating that this fragment must contain the 5′ part of *rrnG* and the 3′ end of *rrnH*, with the intergenic space between them showing that these operons are adjacent (Fig. 3 and Table 1). Moreover, the 2.9-kb

TABLE 1. *EcoRI* rescues of *rrn* operon flanking regions

Plasmid and *rrn* operon[a]	*EcoRI* fragments (kb)	Hybridization[b]	
		5′ 16S	3′ 23S
pWR306, *rrnG*	6,200	–	–
	2,900	+	+
	2,300	–	+
	800	–	–
pWR291, *rrnH*[c]	6,200	–	–
	3,600	+	+
	2,300	–	+
	800	–	–

[a] The inserted plasmid was pGR110.
[b] Probes used were a 1.9-kb *EcoRI* fragment from pGS227 (8) containing the 5′ end of the 16S gene and a 1.2-kb *EcoRI-PstI* fragment from pYR104 containing the 3′ part of the 23S gene.
[c] The integrant strain is missing *rrnG* by spontaneous deletion.

*Eco*RI fragment containing parts of *rrnH* and *rrnG* is the fragment no longer found in the spontaneous deletion strains of BD170 and its relatives (Fig. 3a and b).

Plasmid pWR291 was rescued from *rrnH* in a strain which was already missing the 2.9-kb *Eco*RI fragment. This rescue contains the same 2.3-kb *Eco*RI fragment as pWR306, indicating that the *Eco*RI site normally found downstream of *rrnG* is now next to *rrnH* (Fig. 3b and c and Table 1). A 3.6-kb *Eco*RI fragment was found containing both 16S and 23S rDNA of two adjacent operons, namely the 5′ end of *rrnH* and the 3′ end of *rrnI* and the space between them (Fig. 3a to c and Table 1), demonstrating the presence of a third *rrn* gene set in the cluster. The 2.9-kb *Eco*RI fragment found upstream of the *rrnG* insertion should normally be found downstream of *rrnH* since these operons are contiguous. When *rrnG* was deleted in strain BD170, this 2.9-kb fragment was no longer found (Fig. 3a and b). Instead, the 2.3-kb *Eco*RI fragment was now situated at the 3′ end of *rrnH* (Fig. 3c). Finally, we note that the 3.9-kb fragment no longer found in strain CU420 (Fig. 2b) also hybridized to both 16S and 23S rDNA (Fig. 3a and b). Although a plasmid was not rescued from that region, the result suggests that the 3.9-kb *Eco*RI fragment contains the 3′ end of *rrnJ* and the 5′ end of *rrnW* (Fig. 3c).

DISCUSSION

In this paper we showed that in certain common laboratory strains, many constructed by Dubnau et al. (4), such as BD170 (*trpC2 thr-5*), BD79 (*leuB1 pheA1*), and BD29 (*leuB1 argA2*), or by Ward and Zahler (13), such as CU420 (*trpC2 leuB6 ilvC4*), the number of *Bcl*I *rrn* homologs was 9 instead of 10, revealing in all these cases the loss of either the smallest 4.8-kb or the 5.8-kb fragment, respectively. Earlier studies with the first group of strains (i.e., the BD170 type) showed that genomic digests with other restriction enzymes exhibited the loss of an *Eco*RI 2.9-kb 23S homolog, *Hin*dIII 5.5-kb 16S and 5.1-kb 23S homologs, and *Sma*I 2.1-kb 16S and 2.0-kb 23S homologs (6). Similarly restricting the DNA of another laboratory strain described as 168 *trp* with *Bam*HI, *Hin*dIII, and *Pst*I and probing with a small 16S-specific rDNA fragment, losses of unique 5.3-, 5.3-, and 2.2-kb homologs, respectively, were reported (10). As the authors pointed out, by the time the difference was noted, the parental *trp*$^+$ strain had been lost and was not available for further analysis. Furthermore, a 3.1-kb *Eco*RI rDNA fragment analogous to our 2.9-kb homolog which contains the intergenic spacer region from two tandemly repeated rRNA gene sets was discussed in that report (10). These naturally occurring deletions probably arose via intrachromosomal recombination within each set of tandemly repeated *rrn* genes (*rrnJ-rrnW* and *rrnI-rrnH-rrnG*), and therefore deletions occur only within these gene sets, as was observed in our strain collection of *B. subtilis*. Similarly, Loughney et al. (10) speculated that a recombinational event within the triplet of closely spaced rRNA gene sets led to the deletion they observed.

From the restriction analyses of rescued plasmids pWR306 and pWR291, we propose a model involving homologous recombination between *rrnH* and *rrnG*, leading to the deletion of an *rrn* operon equivalent and the intergenic spacer. As shown in Fig. 5, the model could explain the genomic origin of the 3.6- and 2.3-kb *Eco*RI fragments rescued from pWR291 and the absence of the 2.9-kb *Eco*RI fragment. The model also explains the absence of the 4.8-kb

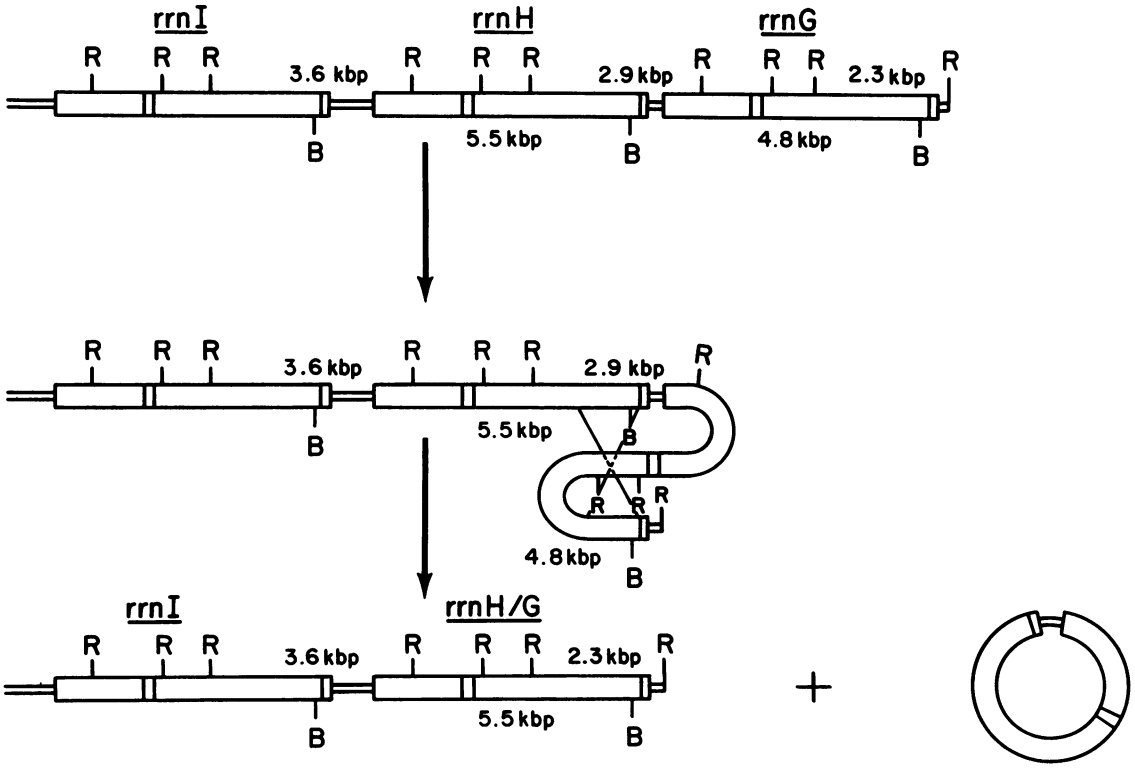

FIG. 5. Model for spontaneous deletion of operon *rrnG* by intrachromosomal recombination. Crossing-over occurs between *rrnH* and *rrnG*, deleting an rRNA gene set equivalent, the intergenic spacer, and creating a fusion operon (*rrnH/G*) made up of the 5′ part of *rrnH* and the 3′ end of *rrnG*. DNA which is looped out is presumably lost. Restriction sites: B, *Bcl*I; R, *Eco*RI.

*Bcl*I homolog and the presence of the 5.5-kb *Bcl*I fragment. Such an intrachromosomal recombination event will result in the formation of a fusion operon, in which the 5′ *Bcl*I and *Eco*RI sites correspond to those of *rrnH* and the 3′ *Eco*RI site corresponds to that of *rrnG* (Fig. 5). An identical event must take place between *rrnJ* and *rrnW*, where a 3.9-kb *Eco*RI fragment or a 5.8-kb *Bcl*I fragment spanning the space between these operons become deleted, leaving a single fused operon. To date we have not rescued the spacer DNA from integrant strains with insertions into the *rrnJ-rrnW* operons, perhaps because it may contain a cluster of tRNA genes (14). Deletion events between *rrnI* and *rrnH* have not been seen, nor have integration events into *rrnI*. Rescued plasmid pWR291 contains the 3.6-kb *Eco*RI fragment which spans the space between *rrnI* and *rrnH* that theoretically should also be deleted by the same mechanism. The presence of six tRNA genes (12, 14) in the intergenic spacer between *rrnI* and *rrnH* may be vital, and any deletions of that region would render the cells inviable.

Physical evidence from Southern hybridizations showed that the 5.8-kb *Bcl*I homolog is the fragment found deleted either spontaneously or during plasmid insertion into the *rrnJ-rrnW* cluster. Similar induced events were described for the triplet *rrnI-rrnH-rrnG*. One may propose, as we did previously (8), that the pathway for inducing such deletions is through the interaction between a concatemer of the plasmid and the tandem repeat of operons. This is shown in Fig. 4 as a general model of integration of a multimeric form of a prototype plasmid containing ribosomal sequences into the *rrnI-rrnH-rrnG* cluster. A double crossover between two repeated *rrn* sequences of the integrable plasmid would generate two missing *Bcl*I fragments and one high-molecular-weight fragment (Fig. 4). In the resulting Cm^r transformant, the 3′ side of *rrnH*, the intergenic spacer, and the 5′ side of *rrnG* are deleted and replaced by the plasmid. Upon *Eco*RI restriction of the chromosomal region having the plasmid, only one fragment containing 23S and 5S rDNA sequences would be produced. The model predicts one *Eco*RI fragment with 16S and 23S homology, and an intergenic spacer is also deleted (Fig. 4). The validity of the model could be further tested by additional rescues of the chromosomal sequences adjacent to the plasmid of such integrant DNAs.

In conclusion, the presence of multiple copies of rRNA genes in procaryotic genomes has implications for providing an avenue for the generation of tandem duplications or deletions of the genetic material lying between any two pairs of *rrn* genes. In enteric bacteria such as *E. coli* K-12 and *Salmonella typhimurium* LT-2, intrachromosomal crossover between either the spacer tRNAs of *rrnD* and *rrnB* or in the 23S region of these two operons has occurred, as reported by Hill and Harnish (7) and by Lehner et al. (9). In *B. subtilis*, deletions involving tandemly arranged *rrn* operons occurred as reported here. As shown in Fig. 5, the DNA which is looped out can either be lost or be involved in the amplification process of *rrn* operons. Finally, we (8) and Loughney et al. (10) found that *B. subtilis* strains with natural *rrn* deletions or those with deletions induced by plasmid insertions reported here grew as well as the wild-type strain 168T and sporulated with similar efficiency (W. Williamson and R. Perez, unpublished results). None of the deletion strains reported here gave rise to cells with altered colony morphology, as reported by Loughney et al. (10), but their strains were not isogenic, and therefore this difference may not be due to the deletion. In *E. coli*, deletion of one of the seven rRNA operons (*rrnE*) failed to cause any decrease in growth rates and RNA synthesis (5). Certainly the high multiplicity of *rrn* operons in bacteria allow for deletions to occur without rendering the cells inviable. It remains to be proven which of the *rrn* operons in *B. subtilis* are indispensable and which are implicated in sporulation and germination functions.

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LITERATURE CITED

1. **Bott, K., G. C. Stewart, and A. G. Anderson.** 1984. Genetic mapping of cloned ribosomal RNA genes, p. 19–34. *In* J. A. Hoch and A. T. Ganesan (ed.), Syntro Conference on Genetics and Biotechnology of Bacilli. Academic Press, Inc., New York.
2. **Chow, T. L., and N. Davidson.** 1973. Electron microscope mapping of the distribution of ribosomal genes of the *Bacillus subtilis* chromosome. J. Mol. Biol. **75**:265–279.
3. **Dedonder, R. A., J. A. Lepesant, J. Lepesant-Kejzlarova, A. Billault, M. Steinmetz, and F. Kunst.** 1977. Construction of a kit of reference strains for rapid genetic mapping in *Bacillus subtilis* 168. Appl. Environ. Microbiol. **33**:989–993.
4. **Dubnau, D., C. Goldthwaite, I. Smith, and J. Marmur.** 1967. Genetic mapping in *Bacillus subtilis*. J. Mol. Biol. **27**:163–185.
5. **Ellwood, M., and M. Nomura.** 1980. Deletion of a ribosomal ribonucleic acid operon in *Escherichia coli*. J. Bacteriol. **143**: 1077–1080.
6. **Gottlieb, P., G. LaFauci, and R. Rudner.** 1985. Alterations in the number of rRNA operons within the *Bacillus subtilis* genome. Gene **33**:259–268.
7. **Hill, C. W., and B. W. Harnish.** 1982. Transposition of a chromosomal segment bounded by redundant rRNA genes into other rRNA genes in *Escherichia coli*. J. Bacteriol. **149**:449–457.
8. **LaFauci, G., R. L. Widom, R. L. Eisner, E. D. Jarvis, and R. Rudner.** 1986. Mapping of rRNA genes with integrable plasmids in *Bacillus subtilis*. J. Bacteriol. **165**:204–214.
9. **Lehner, A. F., S. Harvey, and C. W. Hill.** 1984. Mapping and spacer identification of rRNA operons of *Salmonella typhimurium*. J. Bacteriol. **160**:682–686.
10. **Loughney, K., E. Lund, and J. E. Dahlberg.** 1983. Deletion of an rRNA gene set in *Bacillus subtilis*. J. Bacteriol. **154**:529–532.
11. **Stewart, G., and K. Bott.** 1983. DNA sequence of the tandem ribosomal RNA promoter for *B. subtilis* operon *rrnB*. Nucleic Acids Res. **11**:6289–6300.
12. **Vold, B.** 1985. Structure and organization of genes for transfer ribonucleic acid in *Bacillus subtilis*. Microbiol. Rev. **49**:71–80.
13. **Ward, J. A., and S. A. Zahler.** 1973. Genetic studies of leucine biosynthesis in *Bacillus subtilis*. J. Bacteriol. **116**:719–726.
14. **Wawrousek, E. F., and J. N. Hansen.** 1983. Structure and organization of a cluster of six tRNA genes in the space between tandem ribosomal RNA gene sets in *Bacillus subtilis*. J. Biol. Chem. **258**:291–298.