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Factors determining frequency of plasmid cointegration mediated by insertion sequence IS*1*

(IS*1* and Tn9 mutants/complementation analysis/amounts of IS*1* proteins/position effect/preferential binding)

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ABSTRACT **We demonstrate that mutants with deletions at either end of the insertion sequence IS*1* lose the ability to mediate cointegration of two plasmids, whereas mutants with deletions or an insertion within IS*1* can mediate cointegration at a reduced frequency. These results, together with the nucleotide sequence analysis of the IS*1* mutants, indicate that the two ends of IS*1* (*insL* and *insR*) and two genes (*insA* and *insB*) that are encoded by IS*1* are required for cointegration. Using a plasmid carrying two copies of IS*1*, we found that the individual IS*1*s mediate cointegration at different characteristic frequencies, and that each of two parts of plasmid DNA segments flanked by the two IS*1*s is a transposon, mediating plasmid cointegration at a unique frequency. When one IS*1* was replaced with a mutant IS*1*, the remaining wild-type IS*1* complemented the cointegration ability of the mutant IS*1* as well as a resulting mutant transposon that was then flanked by a wild-type IS*1* and a mutant IS*1*. The efficiency of this complementation reflected the characteristic ability of an individual IS*1* present on the plasmid to promote cointegration. The results suggest that the IS*1*-encoded proteins are produced in different amounts, depending on the location of IS*1* in the plasmid, and that these amounts determine the efficiency of complementation of the cointegration ability of a mutant IS*1* as well as a mutant transposon. However, the location of an individual IS*1* itself can also determine the frequency of cointegration in the presence of a given amount of the IS*1* proteins. On the basis of the observation that the cointegration ability of a mutant IS*1* is less efficiently complemented than is the ability of a mutant transposon, we also suggest that the IS*1*-encoded proteins can function in *trans*, but act preferentially on the IS*1* or transposon sequence from which they are produced in promoting cointegration.**

IS*1* is a DNA element, 768 base pairs (bp) in length, that translocates from one site to another (1–3). It has been shown that two IS*1* sequences can translocate together with the DNA segment flanked by them. For example, the chloramphenicol transposon Tn9 is flanked by two IS*1*s (4). IS*1* can also mediate cointegration between two plasmids, if one plasmid contains a copy of IS*1*. The resulting cointegrates have been shown to contain direct repeats of IS*1* at the junctions between the two parental plasmid sequences (5).

Analysis of the nucleotide sequence of IS*1* showed that it has an inverted repeat of length approximately 35 bp at its ends (3, 6–9). Recent comparisons of the sequence of IS*1* and isoinsertion sequences of IS*1* from several species of *Shigella* led us to propose the existence of two translation reading frames within IS*1* (designated as *insA* and *insB*) encoding 91 and 125 amino acid polypeptides, respectively (10).

In this paper, we report the isolation of deletion and insertion mutants of IS*1* that affect the frequency of IS*1*-mediated plasmid cointegration. Analysis of the nucleotide sequence and of complementation of these mutants will elucidate the sites and genes of IS*1* as well as their functions in cointegration.

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MATERIALS AND METHODS

Bacteria and Plasmids. The *Escherichia coli* strains JE5507 and its *recA*$^-$ derivative JE5519 and the plasmids pHS1, pMZ71, and ColE1, were described previously (5, 11). pMZ71 is an insertion mutant of pHS1 and carries a copy of IS*1* (11). The pYM and pCF plasmids will be described in *Results*. Phage P1Cm0c225 (P1Cm) was supplied by S. Iida. Plasmid DNAs were prepared according to Ohtsubo *et al.* (12).

Enzymes. The restriction endonucleases *Bst*EII (New England BioLabs), *Pst* I, *Eco*RI, *Sma* I, *Sst* II (Bethesda Research Laboratories), and *Tth* 111I (supplied by T. Shinomiya), were used. The reaction conditions for these enzymes were as recommended by the supplier or *Tth* 111I was used as described in ref. 13. Phage T4 DNA ligase (Bethesda Research Laboratories), S1 nuclease (Miles), and Klenow fragments of *E. coli* DNA polymerase I (Boehringer Mannheim) were also used.

Crude Lysis. A simple way to isolate the plasmid DNA in bacterial cells and to examine the DNA with restriction enzymes is the crude lysis method. The method is described elsewhere (14).

Construction of IS*1* Mutants. pMZ71 was digested with either *Pst* I or *Bst*EII. Because pMZ71 has two *Tth* 111I cleavage sites, pMZ71 DNA was partially digested with this enzyme to obtain the full-length linear DNA, which was separated in 0.7% agarose gel and eluted electrophoretically. After extractions with phenol and ether and precipitation with ethanol, the DNA (2 μg) was treated with 400–1200 units of S1 nuclease under the conditions described (15). After phenol treatment and dialysis against 10 mM Tris·HCl (pH 8.0)/0.1 mM EDTA, ligation was carried out in 20 μl of 60 mM Tris·HCl (pH 8.0)/10 mM $MgCl_2$/10 mM dithiothreitol/1 mM ATP for 16 hr at 14°C with 10 units of T4 DNA ligase. The ligated DNA was used for transformation according to the method essentially described by Mandel and Higa (16). As will be described in the text, an insertion mutant of IS*1* was also isolated by using DNA polymerase I (Klenow) under conditions described in ref. 17. The nucleotide sequences of all of the mutated regions were determined by using the Maxam and Gilbert technique (18).

Construction of pCF Plasmids. To construct pCF71 and pCF71-14, pMZ71 or pYM114 DNA (2 μg) and P1Cm DNA (containing Tn9) (25 μg) were digested with *Pst* I (see Fig. 3). To construct pCF71-3, pYM103 DNA (2 μg) and P1Cm DNA (25 μg) were digested with *Bst*EII. To construct pCF71-314, pCF71-14 and pCF71-3 DNAs were digested with *Eco*RI, which produced two fragments from each, and the smaller fragment (α) of pCF71-14 and the larger fragment (β) of pCF71-3

Abbreviations: bp, base pair(s); kb, kilobase pair(s); Tc, tetracycline; Cm, chloramphenicol.

were then purified by agarose gel electrophoresis. The appropriate fragments from the above digestions were ligated in 50 μl and used for transformation. Plasmid DNA was isolated from a tetracycline (Tc)- and chloramphenicol (Cm)-resistant transformant and was examined for the presence and the orientation of Tn9 by double digestion with *Sst* II and *Bst*EII or *Pst* I.

Analysis of the Frequency of Cointegration. JE5507 and JE5519 cells harboring pMZ71 (or a pYM or pCF plasmid) and ColE1 were grown in 5 ml of L broth at 25°C overnight. Then 0.1 ml of a $1:10^6$ dilution was inoculated into each of 20 test tubes, each containing 5 ml of L broth, and the cells were grown at 25°C for 36–48 hr. Next 0.1 ml of the culture was plated on agar plates containing a high concentration of tetracycline (20–25 μg/ml for JE5507 and 10–15 μg/ml for JE5519) and incubated at 42°C for 24 hr. Under these conditions, cells containing cointegrates can grow, whereas other cells cannot (less than 0.01% survival). However, because the efficiency of plating of cells carrying a typical cointegrate differed slightly in different experiments, the number of cells containing cointegrates was normalized by the efficiency of plating value in each test. The frequency of cointegration was calculated from the number of tetracycline-resistant cells by using the fluctuation test as described (11, 19).

RESULTS

Isolation of IS*1* Mutants. Fig. 1 is a map of IS*1* in the plasmid pMZ71, showing the locations of two possible coding frames (*insA* and *insB*) and two ends (*insL* and *insR*) within IS*1*. As also shown in Fig. 1, pMZ71 has single *Pst* I and *Bst*EII sites, both located within IS*1* (11). pMZ71 contains two *Tth* 111I sites, one of them within IS*1*. After digestion with any one of these enzymes, we introduced deletions around the cleavage site by using S1 nuclease (see *Materials and Methods*). Fig. 1 summarizes the mutant plasmids obtained (designated pYM). The mutant IS*1*s in the pYM plasmids were called IS*1*-*n*, in which *n* represents the last one or two numbers of the plasmid designation. Some mutants had deletions within the *insA* or *insB* coding frames, whereas other mutants had deletions extending into both coding frames or from one coding frame into one of the ends of IS*1* (*insL* and *insR*).

We also isolated two mutant plasmids with deletions outside IS*1*; pYM144 has lost a sequence around the *Tth*111I site outside IS*1*, whereas the pYM711 deletion is in the small *Sma* I fragment of length 196 bp, which lies within IS*102*, another insertion sequence that is present in pMZ71, as will be described later. The insertion mutant (IS*1*-31) was constructed by digestion with *Bst*EII, filling in the single-strand ends with DNA polymerase I, and ligation with T4 DNA ligase. The resulting 5-bp insertion lies between the *insA* and *insB* coding frames, causing no changes in either frame.

Genetic Analysis of the IS*1* Mutants. (i) *Cointegration system used for the analysis.* pMZ71 is an IS*1*-containing derivative of pHS1, a plasmid carrying the Tc resistance gene (11). pMZ71 and pHS1 also contain IS*102* (18) (see Fig. 2). Both IS*1* and IS*102* have been shown to mediate cointegration between two plasmids, resulting in a duplication of the IS sequence that mediates the cointegration event (5, 20). Because pMZ71 carries those two different IS sequences, cointegrates to be formed between pMZ71 and ColE1 can be classified into two types, one mediated by IS*1* and the other mediated by IS*102*, as schematically represented in Fig. 2. These two can be distinguished by using *Pst* I or *Bst*EII, each of which cleaves once within IS*1*, and *Sst*II, which cleaves at one site in IS*102*. The duplicated IS sequence in the cointegrates gives rise to an additional cleavage site for *Pst* I or *Bst*EII for IS*1*-mediated cointegration or *Sst* II for IS*102*-mediated cointegration (Fig. 2; also see refs. 5 and 20).

Because DNA replication of pMZ71 (and pHS1) is temperature sensitive, but that of ColE1 is normal at 42°C, cells carrying cointegrates were able to be selected in the presence of Tc at 42°C from cells harboring pMZ71 (or pHS1) and ColE1, as demonstrated previously (11, 20). As shown in Table 1, the frequency of cointegration (determined as described in *Materials and Methods*) between pMZ71 and ColE1 is approximately 500 times greater than that between pHS1 and ColE1. Table 1 also presents the distribution of the two types of cointegrates as determined by cleavage analysis. When 40 independent cointegrates formed between pMZ71 and ColE1 were analyzed, all of them were found to be mediated by IS*1*, whereas all 21 cointegrates formed between pHS1 and ColE1 were mediated by the IS*102* carried by pHS1.

(ii) *Effect of IS1 mutations on plasmid cointegration.* Table 1 summarizes the frequency of cointegration between ColE1 and each of the pYM plasmids. The plasmids pYM144 and pYM711, which have deletions outside of IS*1*, were able to form IS*1*-mediated cointegrates at almost the same frequency as that between ColE1 and pMZ71 (see Table 1, lines 3 and 4). In contrast, the pYM plasmids with mutations within IS*1* showed less than 1/100th as many cointegrates as between ColE1 and pMZ71 (Table 1, lines 5–9). We examined the structure of the resulting cointegrates by digestion with *Pst* I, *Bst* II, or *Sst* II and found that about half of the cointegrates were mediated by

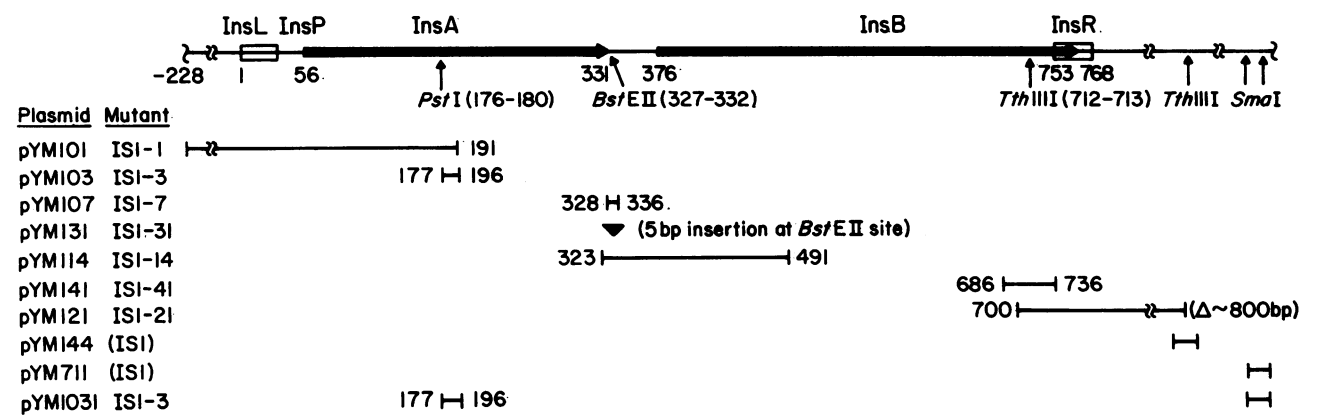

FIG. 1. Map of IS*1* in pMZ71 and the location of the IS*1* mutations. The open boxes represent the inverted repeat sequences at the ends of IS*1*, named *insL* and *insR*. The arrows on the IS*1* map show the location and orientation of the two putative coding frames, *insA* and *insB* (10). *insP* denotes a hypothetical promoter for *insA* and *insB*. All the numbers represent the coordinates to the nucleotide sequence of IS*1* (3). The solid lines below the IS*1* map represent the location of the deletions in IS*1*. The filled triangle in IS*1*-31 represents an insertion.

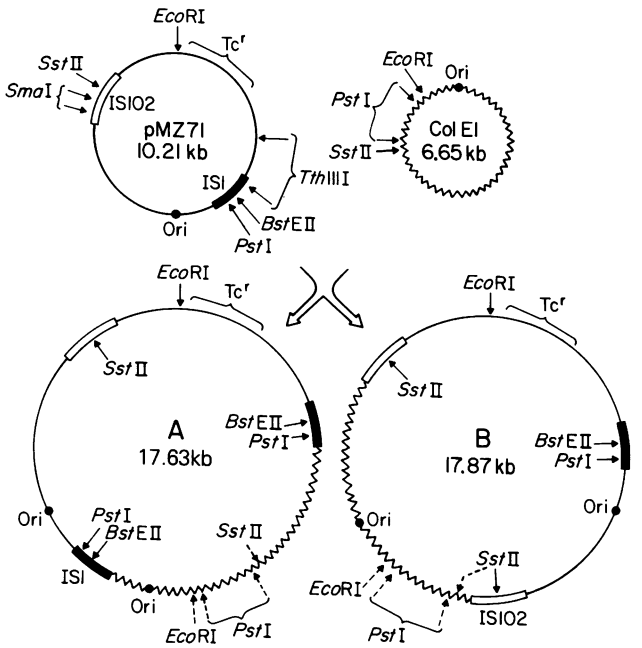

FIG. 2. Structure of two types of cointegrates (A and B) formed between pMZ71 and ColE1. IS*1* (768 bp) and IS*102* (about 1000 bp) (3, 20) are shown by the filled and open boxes, respectively. pHS1 has a structure identical to pMZ71 except for IS*1*. Cleavage sites for restriction enzymes are shown. The integration site of pMZ71 into ColE1 is unique for each cointegrate. Therefore, the *Pst* I and *Sst* II sites on the ColE1 sequence in the cointegrates are shown by the dashed arrows. kb, Kilobase pairs; Ori, origin of replication; Tc^r, tetracycline resistance gene.

Table 1. Frequency of cointegration between ColE1 and various plasmids

Plasmid	Mutation*	Frequency of cointegration per division cycle × 10^8	Number of examined cointegrates mediated by IS*1* (mutants)	Number of examined cointegrates mediated by IS*102*
pMZ71	Wild type	73	40	0
pHS1	No IS*1*	0.15	0	21
pYM144	Wild type†	86	11	0
pYM711	Wild type†	41	12	0
pYM103	*insA*	0.38	7	16
pYM107	*insA*	0.18	6	9
pYM131	Between *insA* and *insB*	0.054	3	12
pYM114	*insA*, *insB*	0.041	1	2
pYM141	*insB*	0.57	3	4
pYM1031	*insA*‡	0.074	2	0
pYM101	*insL*, *insA*	0.069	0	11
pYM121	*insB*, *insR*	0.019	0	10

The frequency was the average of two or three experiments. We used JE5507 for these experiments. Similar results were obtained with JE5519 (*recA*$^-$) (data not shown).
* Location of mutation is shown in Fig. 1.
† The deletion occurred outside of IS*1* (see Fig. 1 and text).
‡ pYM1031 is a derivative of pYM103 and has a deletion in IS*102* (Fig. 1).

the mutant IS*1* and the other half by IS*102*. It should be noted that the *E. coli* K-12 strains used for these experiments contain 10 copies of IS*1* on the chromosome (21). Therefore, we believe that these IS*1*s were able to complement the cointegration ability of the mutant IS*1* on each of the pYM plasmids with a low efficiency. The pYM plasmids examined here contained IS*102*. The products from IS*102*, if any, however, seem not to complement the cointegration ability of the mutant IS*1*, because the pYM1031 plasmid, which contains the IS*1* mutant (IS*1*-3 of pYM103) and also a deletion within IS*102*, was still able to form cointegrates mediated by IS*1*-3 (but not by the IS*102* mutant), as shown in Table 1, line 10.

pYM101 and pYM121 carrying the mutant IS*1*s (IS*1*-1 and IS*1*-21) with deletions extending into either end of IS*1* (*insL* and *insR*) also formed cointegrates with ColE1 at reduced frequencies (see Table 1, lines 11 and 12). In this case, however, all of cointegrates examined were mediated, not by the mutant IS*1*s, but by IS*102*. All of the above results support the previous assumption that two coding frames (*insA* and *insB*) and two sites (*insL* and *insR*) are required for IS*1*-mediated cointegration. However, as will be discussed later, an additional functional site may exist within IS*1*, because insertion mutant IS*1*-31 exhibits such a low frequency (Table 1, line 5).

Complementation Analysis of Mutant IS*1* by Wild-Type IS*1* Present in the Same Plasmid Genome. (i) *Plasmid cointegration system used for the analysis.* The plasmid pCF71 is a derivative of pMZ71 (namely pHS1::IS*1*) and contains the Cm resistance transposon Tn9, which is flanked by two directly repeated IS*1*s. Fig. 3*a* shows the structure of pCF71 as well as the procedure used to construct pCF71 from pMZ71 and phage P1Cm carrying Tn9. pCF71 was able to form cointegrates with ColE1, which were also selected at 42°C in the presence of Tc. Fig. 3*b* shows the resulting cointegrates, which were classified into four different types. The type A plasmid was the cointegrate mediated by the entire Tn9 sequence. Type B and C plasmids were cointegrates mediated by either one of two IS*1*s present on pCF71. (We denote these as IS*1*L and IS*1*H, as shown in Fig. 3. The letters L and H designate the location of the IS*1* that determines low and high frequency of IS*1*-mediated cointegration, respectively, as will be demonstrated below.) Cells harboring type A, B, or C plasmids showed resistance to Cm because they carried the entire Tn9 sequence. The type D plasmid was formed by transposition of the entire pHS1 sequence together with the two IS*1*s of pCF71 into ColE1, eliminating the sequence containing the Cm resistance gene (see Fig. 3). (However, we refer to this plasmid as a cointegrate in this paper.) We name this newly observed transposon Tn*91*. Similar transposition of the outside sequence of Tn9 has been observed (23). The structures of the four types of plasmids were determined by cleavage analysis. In this case, *Eco*RI, which cleaves at the middle of the unique Tn9 sequence (see Fig. 3), was used in addition to *Pst* I, *Bst*EII, and *Sst* II. Fig. 4 *A* and *B* shows the results of cleavage analysis of each type of cointegrate plasmids.

Table 2 summarizes the frequencies of formation of type A, B, C, or D plasmids that are mediated by Tn9, IS*1*L, IS*1*H, or Tn*91*, respectively. It is interesting that the frequency of formation of the IS*1*L-mediated type B plasmid was about 1/20th that of the IS*1*H-mediated type C plasmid. Importantly, we have determined that IS*1*L and IS*1*H have identical nucleotide sequences (see legend to Fig. 3).

(ii) *Complementation analysis.* We introduced a mutant IS*1* by replacing either one or both IS*1*s (IS*1*L and IS*1*H) in pCF71 with IS*1*-3 or IS*1*-14. IS*1*-3 and IS*1*-14 mediated cointegration at a frequency similar to each other but reduced from wild-type IS*1* (Table 1). A derivative of pCF71, called pCF71-3, is a plas-

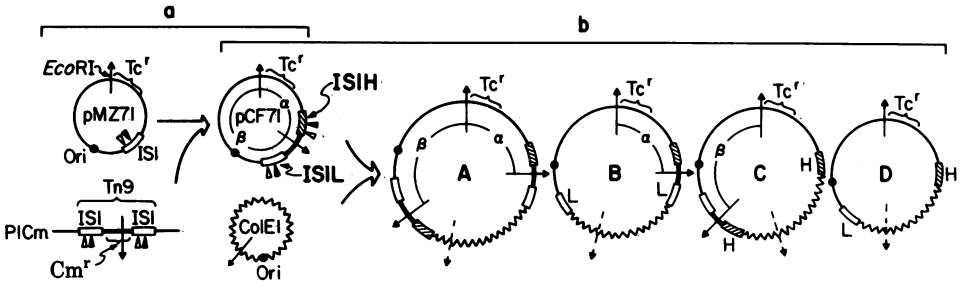

FIG. 3. (*a*) Structure and construction scheme of plasmid pCF71 which carries Tn*9*. Locations of sites for *Pst* I (△), *Bst*EII (▲), and *Eco*RI (↑) are indicated. pCF71 was constructed by inserting the *Pst* I fragment of Tn*9* carried by P1Cm DNA (22) into the *Pst* I site of pMZ71. An important note is that the nucleotide sequence of IS*1* in the *Pst* I or *Bst*EII fragment within Tn*9* of P1Cm was determined to be identical to the IS*1* on pMZ71 (data not shown). pCF71 generates two *Eco*RI fragments, α (4.75 kb) and β (7.32 kb), as indicated. (*b*) Structure of four types of cointegrates and their parents, pCF71 and ColE1. The site of integration of the pCF71 sequences into the ColE1 plasmid is unique for each cointegrate. Therefore, the *Eco*RI sites on ColE1 in the cointegrates are shown by the dashed arrows. Type A plasmid generates two *Eco*RI fragments identical to those of pCF71, α and β. Type B and C plasmids generate an *Eco*RI fragment identical to α and β of pCF71, respectively. See Fig. 4 showing the *Eco*RI digests of these plasmids. Cm^r, chloramphenicol resistance gene.

mid in which IS*1*L has been replaced by IS*1*-3 (designated IS*1*-3L) but it retains a wild-type IS*1* (IS*1*H) (see Table 2). This plasmid thus carries a Tn9 mutant (designated Tn9-3) and a Tn*91* mutant (designated Tn91-3), both of which are flanked by IS*1*-3L and IS*1*H (see Table 2). Another plasmid, pCF71-14, carries a wild-type IS*1* (IS*1*L) and a mutant IS*1* (IS*1*-14H) that is derived from IS*1*-14. pCF71-14 thus carries a Tn9 mutant (Tn9-14) and a Tn*91* mutant (Tn91-14), both of which are flanked by IS*1*L and IS*1*-14H. pCF71-314 carries two mutant IS*1*s, IS*1*-3L instead of IS*1*L and IS*1*-14H instead of IS*1*H, thus carrying a Tn9 mutant (Tn9-314) and a Tn*91* mutant (Tn91-314).

As shown in Table 2 (line 2), the plasmid pCF71-314, carrying two IS*1* mutants, formed cointegrates at frequencies less than 1/100th of those obtained with pCF71 and ColE1. It is interesting that IS*1*-3L was able to mediate cointegration to form the type B plasmid at a lower frequency than that mediated by IS*1*-14H to form the type C plasmid. This is reminiscent of the fact that in the original IS*1*s in pCF71, IS*1*L mediated cointegration at a lower frequency than IS*1*H. The results suggest that the location of IS*1* within a plasmid genome is itself one of the important factors in determining the cointegration frequency, because the IS*1* specific proteins responsible for cointegration in this case are produced from IS*1*s in the chromosome and presumably have the potential to interact (in *trans*) with any IS*1* mutants to promote cointegration with a low efficiency.

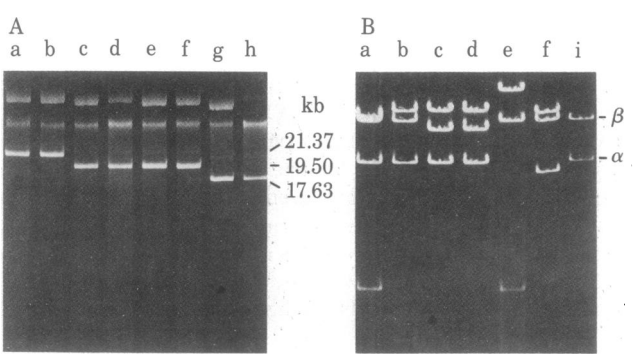

FIG. 4. (*A*) A 0.7% agarose gel showing closed and open circular forms of cointegrates of pCF71 with ColE1. Four pairs (a, b), (c, d), (e, f), and (g, h) are type A, B, C, and D plasmids, respectively (see Fig. 3). The DNAs here were prepared by the crude lysis method. Molecular lengths of closed circles are indicated. Common faint bands are contaminating chromosomal DNA. (*B*) A 0.7% agarose gel showing *Eco*RI digests of the cointegrates shown in *A*. The digestion of type A (a, b) gave rise to four fragments, two of which were identical to the two *Eco*RI fragments, α and β (see Fig. 3 and its legend), of parental pCF71 in lane i. The type B cointegrates (c, d) generated three fragments, one of which was identical to the small *Eco*RI fragment α of pCF71, whereas the type C cointegrates (e, f) generated three fragments, one of which was identical to the large *Eco*RI fragment β of pCF71.

The results of analysis of cointegration of pCF71-3 and of pCF71-14 with ColE1 showed that all four plasmid types were formed at a significantly higher frequency than those formed between pCF71-314 and ColE1 (see Table 2, lines 3 and 4). For cointegration of pCF71-3 with ColE1, the frequencies of formation of type A plasmids mediated by Tn9-3 and of type D mediated by Tn*91*-3 were about the same as observed from the A and D plasmids, respectively, for cointegration between pCF71 and ColE1. Note that both Tn9-3 and Tn*91*-3 contain wild-type IS*1*H, which mediated cointegration at a higher frequency than did IS*1*L. In contrast, the formation of type A and D plasmids mediated by Tn9-14 and Tn*91*-14, respectively, through cointegration between pCF71-14 and ColE1, showed frequencies decreased from those observed between pCF71 and ColE1 (20–30% of the original level). Note in this case that Tn9-14 and Tn*91*-14 contained the wild-type IS*1*L as a component, which mediated cointegration at a lower frequency than IS*1*H. A possible explanation of these results is that IS*1*H, which shows a greater proficiency in cointegration, produces a higher amount of the IS*1*-encoded proteins than IS*1*L does.

For the formation of type B and C plasmids, wild-type IS*1*H was able to complement the cointegration ability of IS*1*-3L, at an efficiency about 30% of the original level (i.e., 1.9×10^{-8} versus 7.1×10^{-8}; see Table 2, lines 3 and 4). In contrast, wild-type IS*1*L was able to complement the cointegration ability of IS*1*-14H at only about 4% of the original level (i.e., 2.1×10^{-8} versus 4.5×10^{-7}; see Table 2, lines 3 and 4). These results support our hypothesis that different amounts of IS*1*-encoded proteins are produced by IS*1*H and IS*1*L, and that the amount of the IS*1*-encoded proteins is another one of the important factors in determining the complementation efficiency for cointegration. However, it should be pointed out that the complementation frequencies observed for formation of type B and C plasmids mediated by IS elements are much lower than the frequencies of formation of type A and D plasmids mediated by mutants of Tn9 and Tn*91*. This suggests that IS*1*-encoded proteins that are produced from wild-type IS*1*, present as a component of a Tn element, act preferentially on the Tn element, thus causing efficient Tn-mediated cointegration. As stated earlier, recombination mediated by Tn*91* or its mutants is not actually cointegration but a transposition event. However, the important point is that both transposition of Tn*91* mutants and cointegration mediated by Tn9 mutants are dependent upon the

Table 2. Frequency of formation of cointegrates between ColE1 and each of pCF plasmids

Plasmid	IS*1*s in plasmid	Frequency of cointegration per division cycle × 10^8			
		A	B	C	D
pCF71	IS*1*L, IS*1*H	22 (Tn*9*)	2.6 (IS*1*L)	54 (IS*1*H)	25 (Tn*91*)
pCF71-314	IS*1*-3L, IS*1*-14H	<0.028 (Tn*9*-314)	0.028 (IS*1*-3L)	0.25 (IS*1*-14H)	0.13 (Tn*91*-314)
pCF71-3	IS*1*-3L, IS*1*H	21 (Tn*9*-3)	1.9 (IS*1*-3L)	45 (IS*1*H)	31 (Tn*91*-3)
pCF71-14	IS*1*L, IS*1*-14H	3.8 (Tn*9*-14)	7.1 (IS*1*L)	2.1 (IS*1*-14H)	12 (Tn*91*-14)

Plasmid types A–D are described in Fig. 3. The frequency of formation of the type D plasmid was obtained by scoring for the Tc-resistant but Cm-sensitive colonies, using a replica plating method. The frequency of formation for the other type of plasmids was obtained as follows: We first collected about 50 independent Tc- and Cm-resistant colonies, each of which was from a different independent culture, and then examined the type of each cointegrate by size and restriction endonuclease cleavage analyses (see Fig. 4). By knowing the distribution of different types of cointegrates, the frequency of formation of each type of plasmid was calculated from the frequency of the formation of the colonies resistant to both Tc and Cm. (IS*102*-mediated cointegrates of pCF71-314 and pCF71-14 with ColE1 are not shown here, because the subject is unnecessary in the present section.) These experiments were done in strain JE5519 (*recA*$^-$).

wild-type IS*1* component. We assume, therefore, that the dependency is due to the characteristic function of IS*1* proteins acting preferentially at the individual IS*1* from which they are produced.

DISCUSSION

The results of genetic analysis of mutant IS*1*s indicate that the position of IS*1* on a plasmid genome and the amount of IS*1* proteins encoded by the *insA* and *insB* coding frames in IS*1* locating at the position are two important factors in determining the frequency of cointegration mediated by IS*1*. Our results also show that the IS*1*-encoded proteins can act preferentially on the IS*1* DNA from which the proteins are produced to promote plasmid cointegration, although they can also act on the other IS*1* DNA, but apparently less efficiently. We and others have previously suggested the possibility of preferentially (or *cis*-) acting transposition enzymes produced by IS*1* and Tn elements such as Tn*10* and Tn*903* (11, 24, 25). Interesting is the effect of position on IS*1*-mediated cointegration. Our recent genetic analysis (unpublished) suggests that transcriptional read-through of the Cm resistance gene affects the expression of the IS*1*L. Details of this analysis will be reported elsewhere.

The reduced effect, reported in the text, of the multiple chromosomal copies of IS*1*s for complementation of cointegration of mutant IS*1*s on a plasmid may be due to one or several possibilities: (*i*) the chromosomal IS*1*s give reduced quantities of proteins, or (*ii*) proteins altered in sequence are less active; (*iii*) the IS*1* proteins are more active on the DNA from which they are produced.

Cointegration mediated by IS*1* is probably a complicated process involving several molecular events (11, 26). We assume that proteins encoded by *insA* and *insB* have at least the function of recognizing the inverted repeat at the end of IS*1* (*insL* and *insR*), because mutant IS*1*s that delete either inverted repeat cannot mediate cointegration.

Nucleotide sequence analysis of IS*1* and its isoinsertion sequences has led us to assume that the mRNA for the *insA* and *insB* proteins is synthesized polycistronically from a promoter (we call *insP*) that is located upstream of the *insA* coding frame (10) (see Fig. 1). In view of this assumption, our finding that an IS*1* mutant (IS*1*-31) with a 5-bp insertion between the *insA* and *insB* coding frames reduced the frequency of cointegration is important. This suggests that the insertion either disturbed synthesis of the portion of the polycistronic message coding for the *insB* protein or disrupted a second independent promoter that may exist between the *insA* and *insB* coding frames. Further analysis of transcription is required to determine whether *insA* and *insB* have separate promoters.

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