JOURNAL OF BACTERIOLOGY, Oct. 1977, p. 332-340

Vol. 132, No. 1
Printed in U.S.A.

# Studies of Colicin E1 Plasmid Functions by Analysis of Deletions and TnA Insertions of the Plasmid

JOSEPH INSELBURG

*Department of Microbiology, Dartmouth Medical School, Hanover, New Hampshire 03755*

Received for publication 22 April 1977

The further identification of regions of the colicin E1 plasmid that affect plasmid functions has been achieved by studying deletions and TnA insertions of the plasmid. Colicin production, colicin immunity, relaxation of plasmid deoxyribonucleic acid, and plasmid incompatibility functions have been examined. A strong correlation has been observed between the ability of colicin E1 plasmid deoxyribonucleic acid to be relaxed and the ability of that plasmid to be transferred by conjugation.

In previous work, TnA transposon insertions of colicin E1 (ColE1) plasmids were isolated and mapped, and their effects on gene expression were examined (12, 19; D. Sherratt, personal communication). In the accompanying paper, deletion mutants of TnA carrying ColE1 plasmids were physically characterized by comparing restriction endonuclease fragments generated by the plasmid (14). In this paper the effects of those deletion and insertion mutants (12) on a variety of ColE1-related properties are examined.

A bacterial cell containing the ColE1 plasmid deoxyribonucleic acid (DNA) ($4.2 \times 10^6$ daltons) can be induced to produce colicin E1 and is immune to the lethal action of that colicin in its environment. In previous work, insertion mutants affecting colicin production (12, 19; Sherratt, personal communication) and colicin immunity (12) were described. In this report the location of such mutants will be examined further.

The ColE1 plasmid is stably maintained in multiple copies in *Escherichia coli*. Depending on growth conditions (5), varying amounts of the covalently closed circular (CCC) plasmid DNA molecules can be isolated as part of a protein complex, the "relaxation complex" (3). When the complex is exposed to a variety of protein-denaturing conditions (4), a site-specific single-strand break, a "relaxation nick," is introduced into the CCC DNA, converting it to open circular (OC) DNA. The location of the relaxation nick site (15, 20) in the vicinity of the ColE1 replication origin (11, 16, 21) has stimulated speculation that DNA "relaxation" is related to plasmid replication. The recent isolation of TnA insertion mutants of ColE1 that inhibit relaxation suggested that, although a plasmid-directed function was involved in relaxation, DNA relaxation was apparently not essential for plasmid replication (12). In this report a further examination of mutants affecting relaxation will be described. A correlation between the Hfr-mediated conjugational transfer of ColE1 plasmid DNA and relaxability of plasmid DNA will be reported. A possible role of relaxation in replication associated with the conjugational transfer of DNA will be considered. The ability of wild-type ColE1 to complement the transfer of mutants of the ColE1 plasmid will be reported.

It was previously shown that the frequency of the stable establishment of a conjugationally transferred ColE1 DNA in a recipient cell carrying a ColE1 derivative was reduced by a factor of 5- to 10-fold (10). Experiments demonstrating the phenomenon of incompatibility among ColE1 plasmids will be described here, and the detection of a region of the ColE1 genome that affects incompatibility will be reported.

## MATERIALS AND METHODS

**Bacterial strains and plasmids used.** The *E. coli* K-12 strains P678-54 (10) and HfrH5 obtained from B. Bachmann and their plasmid-carrying derivatives isolated by transforming those strains with purified plasmid DNA (6) were used. The plasmids and some of their characteristics are summarized in Table 1. A map of the extent of deletions of ColE1 and the location of TnA insertions previously published are included to facilitate the interpretation of results (Fig. 1).

**Media and solution.** M9 glucose and glycerol medium was prepared as previously described (12), and Difco nutrient broth and nutrient agar were used. Top agar was 0.7% agar. All sucrose solutions were prepared (wt/vol) in 0.05 M tris(hydroxymethyl)aminomethane (pH 7.0)-0.005 M ethylenediaminetetraacetic acid-0.05 M NaCl.

**Test of colicin production.** Colonies grown on

TABLE 1. *Summary of the properties of plasmids used in this study*[a]

| Plasmid insertion or deletion | Colicin production | Colicin immunity | DNA relaxation | Conjugational transfer | ColE1 incompatibility | Mol wt of ColE1 ($\times 10^6$) | Total mol wt of ColE1 + TnA ($\times 10^6$) |
|---|---|---|---|---|---|---|---|
| Insertion | | | | | | | |
| 6-12 | − | + | + | + | Low | 4.2 | 7.0 |
| 5-42 | − | + | + | + | Low | 4.2 | 7.0 |
| 2-35 | − | + | + | + | Low | 4.2 | 7.0 |
| 7-6 | − | + | + | + | Low | 4.2 | 7.0 |
| 3-1 | + | + | + | + | NT | 4.2 | 7.0 |
| 3-12 | + | + | − | − | NT | 4.2 | 7.0 |
| 0-1 | + | + | − | − | NT | 4.2 | 7.0 |
| 2-14 | + | + | − | − | NT | 4.2 | 7.0 |
| 7-12 | + | + | + | + | NT | 4.2 | 7.0 |
| 6-30 | + | + | + | + | NT | 4.2 | 7.0 |
| Wild-type ColE1 | + | + | + | + | Very high | 4.2 | 7.0 |
| Deletion | | | | | | | |
| 6-30:d6-6 | − | − | − | − | Low | 0.63 | 3.43 |
| 6-30:d2-3 | − | − | − | − | Low | 0.63 | 3.43 |
| 6-30:d1-4 | − | − | − | − | NT | 0.83 | 3.63 |
| 6-30:d6-4 | − | − | − | NT | Low | 1.47 | 4.27 |
| 6:30:d6-4:d55 | − | − | − | − | NT | 1.47 | 2.40 |
| 6-30:d1-3 | − | + | − | NT | NT | 2.03 | 4.83 |
| 6-30:d6-1 | − | − | + | −[b] | NT | 2.18 | 4.98 |
| 6-30:d6-3 | − | − | + | + | Very high | 2.30 | 5.10 |
| 6-30:d6-5 | − | − | + | + | Very high | 2.30 | 5.10 |
| 6-30:d3-1 | − | − | + | + | High | 2.35 | 5.15 |
| 6-30:d2-7 | − | − | + | NT | High | 3.39 | 6.19 |
| d5-36 | + | + | − | − | NT | ≈3.00 | 5.80 |
| d0-11 | − | − | − | NT | NT | ≈1.28 | 3.15 |

[a] The properties listed are compiled from data presented in references 12 and 14 and this paper. Molecular weight estimates determined by agarose gel electrophoresis measurements of endonuclease-generated fragments are approximately accurate to ±5%. NT, Not tested. For more accurate values for relaxation, see reference 12 and Table 2. For more accurate values for measurements of incompatibility, see Tables 4 and 5. Molecular weights are taken from reference 11.

[b] Strains carrying 6-30:d6-1 show altered growth characteristics, therefore making the evaluation of conjugational transfer frequencies difficult.

nutrient agar were chloroformed and overlaid with a colicin-sensitive strain in top agar. The killing of cells above a colony was interpreted as colicin production.

**Test of colicin immunity.** A nutrient agar plate containing a confluent growth of a colicin-producing strain was chloroformed and overlaid with 5 ml of nutrient top agar. A 0.02-ml amount of $1 \times 10^8$ cells per ml was spotted on that plate and on a control plate without a colicin-producing strain. Immune cells gave confluent growth.

**Sucrose density gradient centrifugation.** Sucrose gradients (5 to 20% and 15 to 50%) were prepared and centrifuged at 40,000 rpm at 15°C for 2.5 to 4 h, depending on the sample, in an SW50.1 Spinco rotor and an L3-50 ultracentrifuge. Fractions were collected by drop collection from the bottom of the tube.

**Studies of DNA relaxation.** All plasmid-carrying strains were grown in M9 glycerol to enhance the formation of relaxation-complexed DNA (16). When cells reached about $1.5 \times 10^8$ cells per ml, [$^3$H]thymidine was added at a specific activity of 5 $\mu$Ci of thymidine per $\mu$g and incubated for two generations. The cells were then lysed by using Triton X-100 in the "cleared lysis procedure" as previously described (12). The cleared lysates were immediately frozen and stored at −20°C overnight. $^{14}$C-labeled ColE1 DNA was used as a sedimentation marker. In several experiments a plasmid was labeled not only with [$^3$H]thymidine but also with [$^{14}$C]thymidine during extended replication in chloramphenicol (2). The use of these $^{14}$C-labeled sedimentation markers rather than ColE1 DNA provided no advantage in the interpretation of results. The cleared lysates were treated for 15 min at 37°C with 100 $\mu$g of predigested Pronase per ml and 0.5% sodium dodecyl sulfate prior to layering a 0.2-ml sample on a 15 to 50% sucrose gradient and subsequent centrifugation. The conversion of CCC to OC DNA was used as the criterion for relaxation.

**Conjugational transfer of plasmid DNAs.** The donor strains were all derived from HfrH5 by transforming the cell with plasmid DNA, selecting either Ap$^r$ or colicin immunity, and then demonstrating that the plasmid content of the transformant was identical to the transforming plasmid by isolating the plasmid DNA and comparing it on agarose gels with the original transforming DNA (12). The recipient strains were either P678-54, P678-54 (ColE1), or P678-54, carrying ColE1 with TnA as insertions or a ColE1 deletion. Cells were grown overnight in nutrient broth at 37°C, diluted 1/100 in nutrient broth in the morning, and grown to about $2 \times 10^8$ cells per ml. Sterile trypsin (200 $\mu$g/ml) was mixed with the cells, and 0.1 ml of the donor and 0.1 ml of the recipient were mixed and incubated with very gentle shaking for 3 h. The conjugational mixtures were diluted, mixed for 2 min at maximal speed on

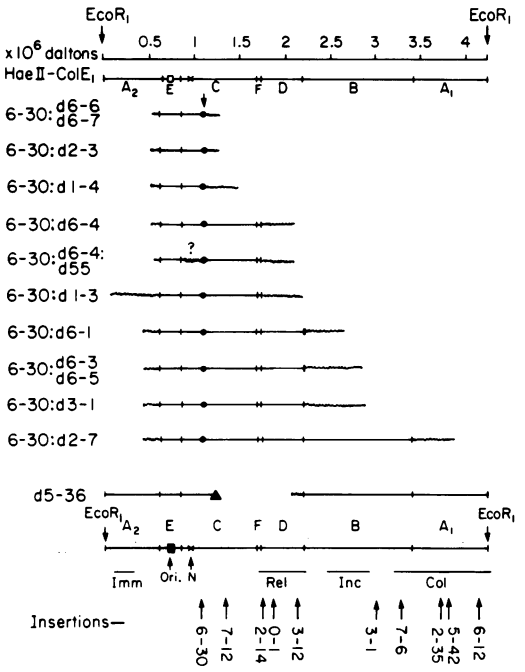


FIG. 1. *Deletions and TnA insertions of ColE1. The HaeII restriction endonuclease map of ColE1 (18; Ohmori and Tomizawa, personal communication) is presented. Cleavage at the single EcoRI endonuclease-sensitive site provides an internal reference. Measurements are $\times 10^6$ daltons.* ● *represents the location of the TnA insertion in the 6-30-derived deletions (14).* ∿∿ *represents portions of partially digested HaeII fragments formed during deletion formation that are fused together in the circular molecules. The relative contribution of DNA from each partially digested fragment is not known in many instances, although estimates of limits were possible (14). The sum of the lengths of fused fragments reflects the total length of that DNA as determined by agarose gel electrophoresis (14) (i.e., the relative amounts of HaeII A2 and B fragments in 6-30:d6-1 and 6-30:d6-3 are not known).* ▲ *represents the location of the TnA insertion in the d5-36 deletion (14). There is approximately 1.0 $\times$ $10^6$ daltons of DNA deleted, which includes the HaeII D, F, and C fragments as well as part of the TnA.* ↑ *represents the site of TnA inserted in various insertion mutants of ColE1 (12). The location of regions associated with specific plasmid functions that are indicated are based on the locations of insertions and the possible extent of deletions affecting the function. The precise limits of such regions are not given by the diagram.* ■ *represents the approximate location of the replication origin.* × *represents the approximate location of the relaxation nick site. Regions affecting various ColE1 functions are: Col, colicin production; Inc, very high levels of incompatibility; Rel, DNA relaxation function; Imm, colicin immunity.*

a Vortex mixer, and plated on nutrient agar with 100 $\mu$g of streptomycin per ml, 100 $\mu$g of ampicillin per ml, both antibiotics, or neither. The viable counts of both donor and recipient were always determined at the time of mixing the cultures, and the total number of recipient cells, as well as the specifically selected recipients, was determined at the end of all conjugations. The efficiency of stable transfer of a plasmid was calculated as the number of recipients carrying the plasmid divided by the total number of recipients. Conjugations were done such that all donors being compared were usually tested at the same time with the same culture of recipient cells to eliminate any variations attributable to the recipient. If particular plasmids were to be tested, control donor and recipient crosses were always performed to insure comparability of results. All conjugation experiments were performed at least three times.

## RESULTS

Deletion mutants of the ColE1 DNA in the ColE1 Ap$^r$ 6-30 plasmid that were generated by exonuclease III digestion of DNA from the *Eco*RI-sensitive site were previously described (12) (Fig. 1). As those deletions were selected on the basis of their Col$^-$ phenotype caused by the loss of DNA around the *Eco*RI-sensitive site (9, 12), little more can be said about the region affecting colicin production in those deletions. Studies of one deletion, d5-36 (12), and the localization of the TnA insertions by restriction endonuclease digestion shed some light on a previous report by So et al. (19) that colicin production is affected by mutants localized between 40 and 46% of the total ColE1 distance from the left end (Fig. 1) of the ColE1 map. That particular region is spanned by the extreme right end of the *Hae*II C fragment, the *Hae*II F fragment, and the left half of the *Hae*II D fragment. d5-36, which is Col$^+$, was found to be missing part of the *Hae*II D fragment, the whole F fragment, and all of the right half of the "C" fragment adjacent to it (see Fig. 1 and reference 14, Fig. 7 and 8). The deletion of that region without leading to a Col$^-$ phenotype suggests that colicin production is not affected by insertions in it. The locations of three insertion mutations (0-1, 2-14, and 3-12) (12) have been corroborated by showing that the *Hae*II D fragment is the only ColE1 *Hae*II fragment missing upon digestion of these plasmids (data not shown). The corroboration of the location of these insertions also strongly suggests that colicin production is not moderated through that region and is only affected by mutations in the right side of the map. Dougan and Sherratt (personal communication, in press) have come to a similar conclusion.

**Colicin immunity.** The deletion mutants 6-30:d1-3 is Imm$^+$. The examination of the d6-30:d1-3 *Hae*II DNA fragment (14) indicated that it contains a new fragment that is a fusion of the *Hae*II D and A2 fragments (see Fig. 2 and Table 1 of reference 14) (Fig. 1). That new fragment contained about 86% of the material in both fragments, indicating that very little of either fragment could have been deleted. As TnA insertions 2-14, 0-1, and 3-12 and the deletion d5-36 all affect the *Hae*II D fragment and are Imm$^+$, it would seem that the information in the D fragment does not affect immunity.

Another deletion, which is Imm$^-$, 6-30:d2-7, is only missing $0.66 \times 10^6$ daltons of DNA from the region around the *Eco*RI site and therefore can only involve the A2 and A1 fragments. Many TnA insertions are located in the *Hae*II A1 fragment, including insertion 6-12, which is located almost at the *Eco*RI site (12). Since none of these insertions, which are all Col$^-$, effects the expression of immunity and since deletion 6-30:d2-7 does, it may be concluded that the information for the expression of colicin immunity is located in the A2 portion of the *Hae*II fragment. These results support the previous prediction that the immunity function is probably located between the Col$^-$ Imm$^-$ insertion mutant 6-32 (12) and the left end of the ColE1 map (Fig. 1). This finding places the immunity region very close to the left end of the map and probably abutting on the region affecting colicin production.

**Relaxation of ColE1 DNA.** It was previously shown that four TnA-carrying plasmids, 2-14, 0-1, 3-12, and d5-36, that also contained an unspecified deletion all inhibited the relaxability of ColE1 DNA. Three of the insertions mapped in or at the boundary of the *Hae*II D fragment, whereas d5-36 mapped in the *Hae*II C fragment by heteroduplex analyses. This finding was confirmed by examining *Hae*II digests of the three insertions (data not shown) and d5-36 (see Fig. 7 and 8 of reference 14). The effect of the TnA insertion d5-36 on relaxation is now understandable in view of the involvement of the *Hae*II D fragment by a deletion carried by that plasmid.

An examination of the relaxability of CCC DNA of all of the deletions isolated was undertaken. It was found that deletions d6-6, d1-4, d6-4, d6-4:d55, and d0-11 gave essentially no relaxation, whereas d1-3 gave a markedly reduced relaxation (Table 2). Representative examples of sucrose density gradients used to determine the relaxation of ColE1 mutants are shown in Fig. 2. All of the deletions that give markedly reduced relaxation have in common the fact that the *Hae*II D fragment is involved in the deletion (Fig. 1). Deletion mutations not affecting the *Hae*II D fragment show essentially normal relaxation. This result further confirms the previous finding that a change involving the *Hae*II D fragment, which is far removed from the relaxation nick site of ColE1, affects the expression of a ColE1 plasmid function required for relaxation but not for replication. As deletion d6-1 shows normal relaxation (Fig. 1), it may be concluded that the transcription of a probable gene product affecting relaxation starts or terminates in the left end of the *Hae*II B fragment.

TABLE 2. *Relaxation of ColE1 DNA*[a]

| DNA | Conversion of CCC to OC DNA by SDS[b] + Pronase treatment (%) |
|---|---|
| 6-30 | 67 |
| 6-30:d6-6 | 0 |
| 6-30:d1-4 | 0 |
| 6-30:d6-4 | 0 |
| 6-30:d6-4:d55 | 0 |
| 6-30d1-3 | 3 |
| 6-30:d6-1 | 56 |
| 6-30:d6-3 | 82 |
| 6-30:d6-5 | 85 |
| 6-30:d3-1 | 26 |
| 0-11 | 0 |
| 5-36 | 3[c] |

[a] All plasmids shown were isolated from P678-54, treated and centrifuged as described in the text. The data were obtained from sucrose gradients similar to those shown in Fig. 2.

[b] SDS, Sodium dodecyl sulfate.

[c] The percent conversion of DNA to the OC form for deletion d5-36 was taken from a previous paper (12) for comparison.

**Conjugational transfer of TnA insertions of ColE1 and ColE1 deletions.** An HfrH strain of *E. coli* was transformed to Ap$^r$ with TnA insertion and deletion plasmids that have been isolated, and the recipients were shown to carry the appropriately sized plasmid DNA. The efficiency of conjugational transfer of these plasmids to *E. coli* strain P678-54 is shown in Table 3. It can be seen that insertions 3-12, 0-1, and 2-14 and deletions d6-6, d6-4, d6-4:d55, d5-36, and d6-1 show a significant reduction in their ability to be transferred. These plasmids, with the exception of d6-1, have in common a block in relaxation of their CCC DNA (Table 2 and reference 11). Although the d6-1 plasmid does not show this characteristic, it was noted that the HfrH derivative grew more slowly than that of the other Hfr isolates. It was initially

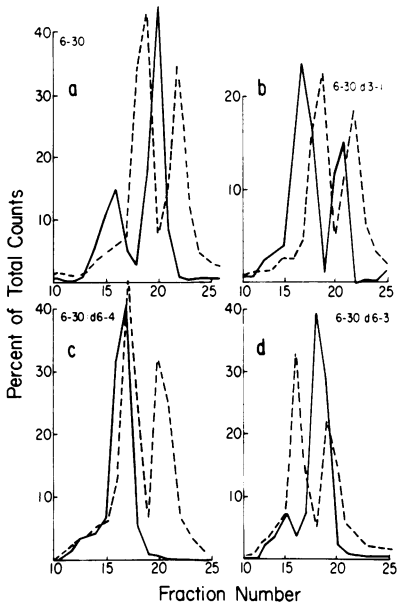


FIG. 2. *Sucrose density gradients of plasmid DNA tested for relaxability. [$^3$H]thymidine-labeled cells carrying deletions of ColE1 plasmid DNA were grown in M9 glycerol and lysed by the cleared lysis procedure using Triton X-100, and the DNA was treated with Pronase and sodium dodecyl sulfate, prior to layering the DNA on 15 to 50% sucrose gradients and centrifuging them at 15°C and 45,000 rpm (189,000 × g) for 3 h. The convertibility of CCC to OC DNA was the criterion for relaxation. In this figure only four representative gradients are shown from which the data in Table 2 were derived. Symbols: - - -, $^{14}$C-labeled ColE1 DNA; ——, $^3$H-labeled ColE1 Ap$^r$ DNAs. The total counts per minute in the samples were (a) 6-30, 18,205 cpm; (b) 6-30:d3-1, 3586 cpm; (c) 6-30:6-4, 7,942 cpm; (d) 6-30:d6-3, 12,108 cpm.*

thought that the selected host was in some way defective. Four other independently isolated HfrH (d6-1) strains were selected, and all of those gave essentially identical results. It is not clear whether plasmid d6-1 transfer reflects a property of the plasmid DNA or an effect the plasmid has on its host. These results will have to be further examined.

**Studies of incompatibility of ColE1 plasmids.** In a previous study it was shown that the presence in *E. coli* of a Col$^-$ mutant of ColE1, caused by a small insertion that maps very near the *Eco*RI-sensitive site, reduced the stable acceptance of conjugationally transferred wild-type ColE1 to that cell by a factor of 5 to 10 (10). As chromosomal gene transfer was shown to be normal, it was concluded that the absence of a stably acquired plasmid was an indication of incompatibility, in which

TABLE 3. *Transfer of ColE1, ColE1 Ap$^r$ insertions, and ColE1 Ap$^r$ deletions to P678-54*

| Donor | Ap$^r$ or Col$^+$ recipients[a] (%) |
|---|---|
| Insertion (HfrH) | |
| 6-12 | 50.0 |
| 5-42 | 52.6 |
| 2-35 | 69.0 |
| 7-6 | 51.3 |
| 3-1 | 44.7 |
| 3-12 | 0.15 |
| 0-1 | 0.003 |
| 2-14 | 0.007 |
| 7-12 | 59.2 |
| 5-36 | 0.01 |
| 6-30 | 56.0 |
| ColE1 | 81.0 |
| Deletion (HfrH) | |
| 6-30:d6-6 | 0.02 |
| 6-30:d6-4 | 0.7 |
| 6-30:d6-4:d55 | 0.007 |
| 6-30:d6-1 | 0.7 |
| 6-30:d6-3 | 50.0 |
| 6-30:d6-5 | 63.0 |
| 6-30:d3-1 | 41.6 |

[a] The percent Ap$_r$ or Col$^+$ P678-54 recipients in each case was calculated as the percentage of total recipients at the end of the conjugation. Conjugation was done as described in the text.

the resident plasmid held a favored competing position. In this series of experiments, the effects of various TnA insertions into ColE1 and deletions of ColE1 were examined for their ability to affect the stable acquisition of ColE1 by cells containing them and the ability of those insertions and deletions to be stably transferred by conjugation to strains carrying wild-type ColE1. An HfrH (ColE1) strain was used to examine the transfer of ColE1 to a variety of P678-54 plasmid-carrying strains (Table 4). The transfer of ColE1 was determined by measuring the percentage of total recipients that had become Col$^+$. Transfer was limited to Col$^-$ recipients. Among the recipients a variety of capabilities for accepting ColE1 was noted. Some strains showed a two- to fourfold reduction in the stable acceptance of ColE1. Two isolates, d6-3 and d6-5, showed more than a 1,000-fold reduction in the stable acceptance of ColE1, and two strains, d3-1 and d2-7, showed a 10- to 100-fold reduction in acceptance.

The conjugational transfer of any of the Ap$^r$ plasmids to P678-54 (ColE1) resulted in fewer than 0.01% of any of the recipients stably maintaining an Ap$^r$ plasmid. Controls done simultaneously showed that all of the Hfr (ColE1 Ap$^r$) cells transferred their plasmids normally to P678-54. These experiments were

repeated on four different occasions, with identical results. As it was possible that the level of Ap$^r$ conferred by the entering plasmid was reached very slowly, recipients were tested for Ap$^r$ after first plating on a nonselective medium or after allowing conjugational cultures, in which the donor was killed by the addition of streptomycin, to grow overnight after dilution to allow a longer period for the expression of the plasmid prior to Ap$^r$ selection. The same failure to obtain Ap$^r$ recipients among the ColE1 recipients was noted. The findings of very high levels of incompatibility exhibited by d6-3 and d6-5 and wild-type ColE1, on the one hand, and the lower level exhibited by other deletions and insertions tested suggest that the DNA carried in d6-3 and d6-5 plasmids affects the expression of information that is necessary for the high level of incompatibility. Insertions located to the right of the DNA contained in d6-3 and d6-5 or deletions of ColE1 DNA to the right of that carried by d6-3 and d6-5 (e.g., 6-30:d3-1 and 6-30:d2-7) reduce the plasmid's expression of incompatibility. How the expression of such a region is influenced by alterations in DNA to the right of it is open to speculation. The two- to fourfold levels of incompatibility among deletions not containing the DNA carried by either d6-3 or d6-5 indicate that a different mechanism of incompatibility may be functioning in those situations. Although the conjugational transfer of Ap$^r$ plasmids to ColE1-carrying recipients was almost undetectable, the transformation of ColE1 cells with Ap$^r$ plasmid DNA was not seriously reduced. It was noted, however, that strains carrying both wild-type ColE1 and ColE1 Ap$^r$ Col$^-$ Imm$^-$ mutants that were constructed by transformation segregated one or the other plasmid at a high frequency if a selection for either colicin immunity or Ap$^r$ was removed (Table 5 and see below). This suggested that the incompatibility expressed between the resident plasmid and entering plasmid was influenced by the mechanism of plasmid entry. The expression of incompatibility made complementation studies difficult to conduct.

TABLE 4. *Conjugational transfer of ColE1 to P678-54 carrying ColE1 (Col$^-$ Ap$^r$) plasmids*

| Recipient | Col$^+$ recipients[a] (%) |
|---|---|
| P678-54 | 67 |
| P678-54 (6-12) | 16 |
| P678-54 (5-42) | 18 |
| P678-54 (2-35) | 25 |
| P678-54 (7-6) | 17 |
| P678-54 (6-30:d6-6) | 30 |
| P678-54 (6-30:d6-4) | 16 |
| P678-54 (6-30:d2-3) | 19 |
| P678-54 (6-30:d1-3) | 20 |
| P678-54 (6-30:d6-3) | <0.02 |
| P678-54 (6-30:d6-5) | <0.01 |
| P678-54 (6-30:d3-1) | 4.5 |
| P678-54 (6-30:d2-7 | 0.6 |

[a] The percentage of Col$^+$ recipients was determined by plating recipient cells on nutrient agar with streptomycin and testing the colonies for colicin production. The donor strain in all cases was HfrH (ColE1). Conjugation was done as described in the text.

**Complementation by ColE1 of conjugational transfer of ColE1 Ap$^r$ mutants.** The previous finding that certain mutants of ColE1

TABLE 5. *Complementation of conjugational transfer of ColE1 Ap$^r$ d6-6 and d6-4 by wild-type ColE1*[a]

| Determination | % of total Ap$^r$ recipients | % of total Col$^+$ recipients | % Col$^+$ | % Ap$^r$ | % of Ap$^r$ that are Col$^+$ |
|---|---|---|---|---|---|
| Donor | | | | | |
| 1. HfrH (d6-6 + E1) | 1.3 | 14.2 | | | |
| 2. HfrH (d6-4 + E1) | 2.7 | 7.4 | | | |
| 3. HfrH (d6-4:d55 + E1) | 5.1 | 5.0 | | | |
| 4. HfrH (d6-4 + E1) | 6.2 | 9.8 | | | |
| 5. HfrH (d6-4 + E1) | 4.5 | 13.2 | | | |
| Donor control | | | | | |
| 6. HfrH (d6-6 + E1) | | | 99.9 | 22 | 70.03 |
| 7. HfrH (d6-4 + E1) | | | 99.7 | 58 | 88.0 |
| 8. HfrH (d6-4:d55 + E1) | | | 97.5 | 22 | 55.9 |

[a] The donor strains were produced by transforming an HfrH (ColE1) strain to Ap$^r$ with DNA from the d6-6, d6-4, or d6-4:d55 Ap$^r$ plasmid. The selection was also for colicin immunity as it was known from incompatibility studies that plasmid segregation occurs when two ColE1 derivatives are in the same cell. The recipient in all cases was P678-54. Hfr colonies picked from an ampicillin plus colicin plate were grown to about $6 \times 10^8$ cells per ml in nutrient broth and plated onto nutrient agar with or without ampicillin at the beginning of the crosses as donor controls (no. 6, 7, and 8). The donor cells shown in no. 6, 7, and 8 were those used in crosses shown in no. 1, 2, and 3. Two other crosses are shown in no. 4 and 5. Ap$^r$ recipients were selected on nutrient agar containing streptomycin and ampicillin. Colicin production was identified with P678-54 or P678-54 (d6-6 Col$^-$ Imm$^-$ Ap$^r$).

were transferred by conjugation at a markedly reduced frequency (Table 3) was further examined by constructing Hfr strains that carried both the ColE1 wild-type plasmid and a ColE1 $Ap^r$ plasmid with a reduced capability to be conjugationally transferred. The question to be asked was whether the ColE1 $Ap^r$ plasmids d6-6, d6-4, and d6-4:d55 show an enhanced conjugational transfer in an Hfr strain also carrying ColE1. The HfrH (ColE1) strain was transformed to $Ap^r$ by ColE1 $Ap^r$ $Col^-$ $Imm^-$ plasmids d6-6, d6-4, and d6-4:d55, and transformants were selected and maintained on agar in the presence of both ampicillin and colicin. The presence of both plasmids was confirmed by isolating and identifying both plasmid DNAs from the donors. These Hfr strains were then inoculated into L broth without any selective pressure and grown to about $6 \times 10^8$ cells per ml without subsequent dilution prior to mixing 1:1 with P678-54 in the presence of trypsin. After 3 h of incubation, the conjugating cells were diluted, broken by shearing, and plated on appropriate plates to test for the transfer of ColE1, the ColE1 $Ap^r$ mutant, or both. Table 5 shows the results of such experiments. It should first be noted that, although 99, 99, and 97% (Table 5, no. 6, 7, and 8) of the Hfr cells grown in nutrient broth and plated on nutrient agar at zero time were $Col^+$, those same cells when simultaneously plated on ampicillin-containing nutrient agar were only 22, 58, and 22% $Ap^r$. Growth in the absence of ampicillin led to a rapid loss of the $Ap^r$ plasmid. Growth in the presence of ampicillin without colicin also led to a significant loss of ColE1 (Table 5, no. 6, 7, and 8). This rapid segregation of plasmids due to incompatibility made the interpretation of complementation potentially difficult as the numbers of donor strains carrying both plasmids was variable, and the transfer of both plasmids to a recipient could lead to a loss of the $Ap^r$ plasmid prior to detectable colony formation. It can be seen, however, that 2.7 and 5.1% of recipients received and retained d6-4 and d6-4:d55 (Table 5, no. 2 and 3) and 1.3% of recipients stably received d6-6 (Table 5, no. 1). In two other experiments, 6.2 and 4.5% of recipients received d6-4 (Table 5, no. 4 and 5). Without any correction being made for the actual number of $Ap^r$ Hfr cells at zero time and the exclusion of $Ap^r$ plasmids from cells also receiving ColE1, it can be seen from Table 5 that the transfer of the $Ap^r$ plasmid was increased from 0.7 (Table 3) to about 5% of recipients for d6-4 (Table 5) and from 0.02 (Table 3) to 1.3% for d6-6 (Table 5). Under these same circumstances, ColE1 was transferred to 7.4% of all recipients from the Hfr (d6-4 + ColE1) and to 14.2% of recipients from the HfrH (d6-6 + ColE1) strains. These are much lower transfer frequencies for ColE1 than are usually seen (40 to 60% of recipients). These results are therefore taken to indicate that the presence of the ColE1 wild-type plasmid in a cell can facilitate the transfer of plasmids d6-6 and d6-4. As it was possible that the $Ap^r$ plasmid transferred at a higher frequency in the presence of ColE1 had recombined with ColE1 and was transferred as a linkage group rather than being transferred because of the presence of a ColE1 gene product, plasmid DNAs were purified from eight $Ap^r$ recipient strains and from eight ColE1 recipient strains of an Hfr (d6-4 + ColE1) cross. The plasmid DNAs obtained from these strains were either d6-4 or ColE1 as determined by agarose gel electrophoresis analysis. In no instance was a molecule of a different size found, which would suggest that recombination had occurred.

## DISCUSSION

The examination of the properties of deletion mutants of ColE1 DNA have substantiated and clarified many of the findings previously reported regarding the localization of various ColE1-related functions. These results are summarized in Fig. 1. The production of colicin is localized to the right side of the genetic map, and no evidence for a second centrally located site affecting colicin production could be found. This finding has been substantiated independently by Sherratt and Dougan (personal communication; Mol. Gen. Genet., in press). The expression of colicin immunity could be localized to the extreme left end of the map (Fig. 1) as was previously postulated (11). Delections affecting the region of ColE1 DNA in which three insertion mutants that inhibit DNA relaxation were mapped also inhibited relaxation. When DNA at a short distance to the right of the *Hae*II restriction-sensitive site between the *Hae*II D and B fragments was present (6-30:d6-1), normal relaxation was noted. This positively localizes plasmid genetic information affecting relaxation to a region between a site around the *Hae*II restriction site between *Hae*II D and B fragments and the region to the left of the 2-14 TnA insertion. Although definitive interpretations of the data dealing with the manifestations of incompatibility exhibited among the ColE1 plasmids are not possible, several points that are worth considering are suggested from the results. Wild-type ColE1 shows a very high level of incompatibility when it is the resident

plasmid in the recipient of a conjugational cross. Deletions d6-3 and d6-5 show a similar level of incompatibility. As the level of incompatibility is as much as 500 times greater in plasmids d6-3 and d6-5 than in smaller plasmids such as d6-1 or d6-4, the argument can be made that a plasmid gene product expression is involved in the incompatibility phenomenon in ColE1. The additional DNA present in plasmids d6-3 and d6-5 and not present in the smaller plasmids is either in the left end of the *Hae*II B fragment or in the right end of the *Hae*II A2 fragment (Fig. 1). The A2-fragment location would appear to be ruled out by the failure of 6-30:d1-3, which probably has much of the A2-fragment DNA, to exhibit very high levels of incompatibility (Table 4). The expression of information in the *Hae*II B-fragment region could be influenced either by the presence of additional DNA found in the d3-1 and d2-7 plasmids or by insertions affecting colicin production in the right end of the plasmid (Fig. 1 and Table 4) if information is expressed from right to left in that part of the map. The presence of a high level and low level of incompatibility further suggests that at least two possible mechanisms could be acting. The further observation that the transfer of $Ap^r$ plasmids to a ColE1-carrying strain by transformation was less affected by the host plasmid than was conjugational transfer suggested that the mechanism of incompatibility was influenced by the route of plasmid entry. The finding that those plasmid mutants that are very inefficiently mobilized for conjugational transfer are precisely those that, by either insertion or deletion mutants, have lost the ability to relax their DNA suggests that the relaxation of the DNA could have a role in the conjugational transfer of DNA. Although relaxation does not seem essential for the plasmid replication within the cell that allows the stable maintenance of the plasmid, it is possible that the conjugational transfer of DNA does require such a relaxation phenomenon. ColE1 was originally shown to replicate in cells as a CCC molecule (7, 13). Although some rolling-circle-type replicative structures of ColE1 have been found, they have not been seen in significant numbers (7). Although this could reflect a bias in molecule selection, I would argue, however, that the rolling-circle structure represents a less frequently used mode of DNA replication that is also involved in the conjugational transfer of ColE1. The rolling circle would require an initial nick (8) that could be provided by the relaxation complex. Evidence exists for the rolling-circle type of transfer of conjugationally transferred DNA (1, 8, 17), but no reports of a role of the relaxation nick have been reported. The result would suggest that, among conjugationally transferable plasmids, a selection for transfer deficiency could turn up relaxation-deficient plasmids. The complementation by wild-type ColE1 of conjugational transfer of mutants showing reduced transferability could be due to the provision by the wild-type ColE1 of a protein providing relaxation. These and other experiments to elucidate this relation must be done.

ACKNOWLEDGMENTS

I thank P. Ware for her excellent technical assistance.

This work was supported by Public Health Service grants AI 08937 and AI 28818 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. **Bonhoeffer, F., and W. Vielmetter.** 1968. Conjugational DNA transfer in *Escherichia coli*. Cold Spring Harbor Symp. Quant. Biol. 33:623-627.
2. **Clewell, D. B.** 1972. Nature of Col $E_1$ plasmid replication in *Escherichia coli* in the presence of chloramphenicol. J. Bacteriol. 110:667-673.
3. **Clewell, D. B., and D. R. Helinski.** 1969. Supercoiled circular DNA-protein complex in *Escherichia coli*: purification and induced conversion to an open circular DNA form. Proc. Natl. Acad. Sci. U.S.A. 62:1159-1166.
4. **Clewell, D. B., and D. R. Helinski.** 1970. Properties of a supercoiled deoxyribonucleic acid-protein relaxation complex and strain specificity of the relaxation event. Biochemistry 9:4428-4440.
5. **Clewell, D. B., and D. R. Helinski.** 1972. Effect of growth conditions on the formation of the relaxation complex of supercoiled ColE1 deoxyribonucleic acid and protein in *Escherichia coli*. J. Bacteriol. 110:1135-1146.
6. **Cohen, S. W., A. C. Y. Chang, and Y. Hsu.** 1972. Nonchromosomal antibiotic resistance in bacteria: genetic transformation of *Escherichia coli* by R-factor DNA. Proc. Natl. Acad. Sci. U.S.A. 69:2110-2114.
7. **Fuke, M., and J. Inselburg.** 1972. Electron microscopic studies of replicating and catenated colicin factor E1 DNA isolated from minicells. Proc. Natl. Acad. Sci. U.S.A. 69:89-92.
8. **Gilbert, W., and D. Dressler.** 1968. Replication of DNA in micro-organisms. Cold Spring Harbor Symp. Quant. Biol. 33:473-484.
9. **Hershfield, V., H. W. Boyer, C. Yanofsky, M. A. Lovett, and D. R. Helinski.** 1974. Plasmid ColE1 as a molecular vehicle for cloning and amplification of DNA. Proc. Natl. Acad. Sci. U.S.A. 71:3455-3459.
10. **Inselburg, J.** 1974. Incompatibility exhibited by colicin plasmids E1, E2, and E3 in *Escherichia coli*. J. Bacteriol. 119:478-483.
11. **Inselburg, J.** 1974. Replication of colicin E1 plasmid DNA in minicells from a unique replication initiation site. Proc. Natl. Acad. Sci. U.S.A. 71:2256-2259.
12. **Inselburg, J.** 1977. Isolation, mapping, and examination of effects of TnA insertions in ColE1 plasmids. J. Bacteriol. 129:482-491.
13. **Inselburg, J., and M. Fuke.** 1971. Isolation of catenated and replicating DNA molecules of colicin factor E1 from minicells. Proc. Natl. Acad. Sci. U.S.A. 68:2839-2842.

14. **Inselburg, J., and P. Ware.** 1977. Isolation and genetic analysis of deletion mutants of colicin E1 plasmids carrying a TnA insertion. J. Bacteriol. 132:321-331.
15. **Lovett, M. A., D. G. Guiney, and D. R. Helinski.** 1974. Relaxation complexes of plasmids ColE1 and ColE2: unique site of the nick in the open circular DNA of the relaxed complexes. Proc. Natl. Acad. Sci. U.S.A. 71:3854-3857.
16. **Lovett, M. A., L. Katz, and D. R. Helinski.** 1974. Unidirectional replication of plasmid ColE1 DNA. Nature (London) 251:337-340.
17. **Ohki, M., and J. Tomizawa.** 1968. A symmetric transfer of DNA strands in bacterial conjugation. Replication of DNA in micro-organisms. Cold Spring Harbor Symp. Quant. Biol. 33:651-657.
18. **Oka, A., and M. Takanami.** 1976. Cleavage map of colicin E1 plasmid. Nature (London) 264:193-196.
19. **So, M., R. Gill, and S. Falkow.** 1975. The generation of a ColE1-Ap$^r$ cloning vehicle which allows detection of inserted DNA. Mol. Gen. Genet. 142:239-249.
20. **Sugino, Y., J. Tomizawa, and T. Kakefuda.** 1975. Location of non-DNA components of closed circular colicin E1 plasmid DNA. Nature (London) 253:652-654.
21. **Tomizawa, J., Y. Sakakibara, and T. Kakefuda.** 1974. Replication of colicin E1 plasmid DNA in cell extracts. Origin and direction of replication. Proc. Natl. Acad. Sci. U.S.A. 71:2260-2264.