JOURNAL OF BACTERIOLOGY, July 1974, p. 144-151

Vol. 119, No. 1
Printed in U.S.A.

# R62, a Naturally Occurring Hybrid R Plasmid

PATRICIA GUERRY, STANLEY FALKOW, AND NAOMI DATTA

*University of Washington School of Medicine, Department of Microbiology, Seattle, Washington 98195, and Royal Postgraduate Medical School, London, United Kingdom*

Received for publication 19 December 1973

R62, a naturally occurring R factor, was shown to be a single deoxyribonucleic acid molecule composed of polynucleotide sequences typical of I group plasmids and also sequences typical of the N group. It determined I pili and belonged to the Iα compatibility group. Although compatible with plasmids of group N, R62 showed complex genetic reactions with N plasmids which are described and interpreted. It is concluded that R62 was the product of illegitimate recombination between an I group and an N group plasmid.

Plasmids of the compatibility groups Iα and N (4, 16) differ in their polynucleotide sequences as shown by lack of hybridization between their deoxyribonuleic acids (DNAs) (13; unpublished observations). R62, a plasmid derived from *Salmonella typhimurium* and conferring resistance to ampicillin (A), streptomycin (S), spectinomycin (Sp), tetracycline (T), and sulfonamides (Su), resembles I plasmids in determining I-type pili (21) and in determining colicine Ib (27). It differs from typical I plasmids in being $fi^+$ (21, 24), and it is unique among plasmids of the I groups in conferring resistance to spectinomycin as well as streptomycin (4) (and, hence, presumably determining streptomycin/spectinomycin adenylate synthetase [11]). Moreover, sulfonamide resistance is not determined by any other known I plasmid. The penicillinase of R62 resembles those of R46 (10), R45, R48, and R205 (19), all of which are N plasmids (15).

Since R62 possessed properties suggesting that it was a product of recombination between an I-group plasmid and an R-factor of some other group, we investigated the compatibility properties and polynucleotide sequence relationships of R62. These studies confirm the impression that R62 is a hybrid plasmid.

## MATERIALS AND METHODS

**Bacterial strains and plasmids.** *Escherichia coli* K-12 strains J53 and J62 (4) were used as host bacteria. The plasmids employed in this investigation are listed in Table 1 together with their relevant genetic characteristics.

**Genetic studies.** The transfer of R factors between strains, tests for incompatibility, and the isolation of plasmid segregants were as described by Coetzee et al. (5). Transduction with phage P1 (23) was performed as described by Clowes and Hayes (4), with tetracycline, streptomycin, or sulfonamide for selection. The presence of I pili was inferred by an increase in titer of phage If1 (25) by using the methods described by Datta et al. (9).

**Characterization of R62 DNA.** Cells of J55 R62 were grown for several generations in 30 ml of minimal salts medium containing [$^3$H]thymine (18 Ci/mmol; New England Nuclear Corp.). The cells were harvested by centrifugation and lysed with Brij 58 (Atlas Chemical Co.) as described by Clewell and Helinski (2). The lysate was centrifuged at 48,000 × *g* for 25 min, and the upper 0.5 ml was removed for the analysis of plasmid DNA. This material (cleared lysate) was diluted 1:1 in TES [0.05 M NaCl, 0.005 M ethylenediaminetetraacetic acid, 0.03 M tris(hydroxymethyl)aminomethane, pH 8] before layering onto 5 to 20% linear neutral and alkaline sucrose gradients together with marker [$^{14}$C]thymine lambda-bacteriophage DNA linear monomers. Neutral sucrose gradients (5 to 20% sucrose in 0.01 M $KPO_4$, 0.05 M NaCl, pH 7) were centrifuged at 200,000 × *g* for 90 min at 30 C in the SW50.1 rotor of a Spinco model L3-50 preparative ultracentrifuge. Alkaline sucrose gradients (5 to 20% sucrose in 0.5 M NaCl, 0.02 M ethylenediaminetetraacetic acid, 0.3 N NaOH, pH 12.5) were centrifuged in the SW50.1 rotor for 20 min at 82,500 × *g* and 25 C. The fractionation of gradients and the counting of fractions were carried out as described previously (12, 28).

Material from Brij-lysed or uniformly [$^3$H]thymine-labeled cells lysed according to method B of Young and Sinsheimer (30) was mixed with concentrated CsCl (American Potash Co.) in TES and ethidium bromide (Calbiochem; final concentration, 250 μg/ml). The final density was adjusted to 1.625 g/cm$^3$, and the mixture was centrifuged for 40 to 60 h at 125,000 × *g* in a fixed-angle type 65 rotor in the L3-50 preparative ultracentrifuge. Ten-drop fractions were collected from a hole punctured in the bottom of the tube. A 10-μliter sample was removed from each

TABLE 1. *Plasmids used in experiments*

| Plasmid | Drug-resistance markers[a] | Compatibility class | Reference source |
|---|---|---|---|
| F | | FI | |
| R1 | A, S, C, Su, K | FII | 21 |
| RTF | | FII | 28 |
| R136 | T | FII | 21 |
| R62 | A, S, T, Su | Iα | 21 |
| R144 | T, K | Iα | 21 |
| ColIb-P9 | | Iα | 16 |
| JR66a | S, K | Iω | 8 |
| N3 | S, T, Su | N | 7 |
| N3T | T | N | 15 |
| R15 | S, Su | N | 7 |
| R390-12[b] | A, S, C, Su | N | 5 |
| R447b | A, K | N | 17 |
| RP4 | A, S, K | P | 9 |

[a] Abbreviations denoted resistance to: A, ampicillin; S, streptomycin; T, tetracycline; Su, sulfonamide; C, chloramphenicol; and K, kanamycin.

[b] Wild-type R390 cells confer resistance to ASTC and Su. In this case, we used a T[s] segregant (5).

fraction and counted as described previously (28). Material from the dense peak containing twisted, covalently closed circular DNA was pooled, and the ethidium bromide was extracted with an equal volume of isopropanol and exhaustively dialyzed.

Purified R62 DNA obtained from cesium chloride-ethidium bromide gradients was examined in an AEI electron microscope by the method of Kleinschmidt (20). Molecules were projected, and their contour lengths were measured with a map tracer. A grating replica (28,000 lines/inch [2.5 cm]) was used as a calibration standard.

Pooled, purified, circular DNA and, where indicated, specific DNA peaks from neutral sucrose gradients were dialyzed into 0.14 M $PO_4$ buffer, pH 6.8 (prepared from equimolar amounts of mono- and disodium phosphate), sonicated to a molecular weight of $2.5 \times 10^5$, denatured by heating at 100 C for 5 min, and used in DNA-DNA duplex studies with other plasmid DNAs, using techniques described in detail previously (13).

## RESULTS

R62 confers resistance to ASTSu and, although it produces I pili and colicin I, it has been classified as $fi^+$. Approximately 0.5% of J53 (R62) colonies isolated on nonselective growth media were either sensitive to streptomycin (retaining ATSu resistance) or to tetracycline (retaining ASSu resistance). Representatives of both the T[s] and S[s] segregants retained the colicinogeny, $fi^+$, and I pilus determinants. The spontaneous rate of loss of all R62 drug-resistance determinants was about 100-fold lower. Phage P1 was propagated on J53 (R62) and used in transduction experiments. The only transductants of R62 obtained were isolated on tetracycline selection plates. These transductants conferred resistance only to tetracycline, but retained the $fi^+$, colicinogeny, and I-pilus determinants of R62 and were transmissible at the same rate as the parental R62 R factor. The transduction experiments suggested, therefore, that either the drug-resistance determinants resided on two distinct plasmids, one of which was nontransducible, or the entire R62 plasmid was too large to be transduced by P1.

The biochemical mechanism of drug resistance mediated by R62 was determined only for ampicillin. Resistance to ampicillin was mediated by a beta-lactamase indistinguishable in its properties from that of R46 (10), using the criteria previously employed by Hedges et al. (18).

**Compatibility and incompatibility of R62 with other R factors.** R62 (and its segregants) did not exclude, were not excluded by, and coexisted stably with the F-like R factors, R1 and R136 (Table 2). R62 was, however, incompatible with I-like R factors (Table 3). R62 in a recipient exerted slight but significant surface exclusion (22, 29) of the prototype Iα group R factor R144, and all of the R144$^+$ exconjugants tested (20 of 20) had lost all recognizable genetic markers of R62. The presence of R144 in a recipient resulted in efficient exclusion of superinfected R62. Exconjugants from this mating had in most cases (35 of 36) lost kanamycin resistance (i.e., had lost R144). The exceptional clone which retained all markers of both R62 and R144 was unstable in the absence of continued selection. After growth on a nonselective medium, 3 of 38 colonies tested had lost all recognizable markers of R62 while retaining those of R144. These data indicated, therefore, that the ASSu markers of R62 were carried on a plasmid of compatibility group Iα.

TABLE 2. *Compatibility of R62 with FII plasmids*[a]

| Donor | Recipient | Frequency of transfer | Transconjugants tested for presence of both plasmids[b] |
|---|---|---|---|
| R53 (R1) | J62 | $2 \times 10^{-2}$ | |
| R53 (R1) | J62 (R62) | $3 \times 10^{-2}$ | 10/10 both present |
| J62 (R62-1)[c] | J53 | $3 \times 10^{-3}$ | |
| J62 (R62-1)[c] | J53 (R136) | $2 \times 10^{-3}$ | 10/10 both present |

[a] Mating mixtures were incubated for 1 h and spread on plates which selected for the nutritional requirement of the recipient and the antibiotic resistance of the donor.

[b] All resistance markers of both plasmids were stably inherited in the transconjugants and separately transmissible from them. R1 was transferred from the double at a frequency of $5 \times 10^{-4}$, i.e., 250-fold reduction due to the $fi^+$ character of R62.

[c] R62-1 was a tetracycline-sensitive segregant of R62.

Since both R144 and R62 carried $T_R$ determinants, incompatibility studies were also carried out between R62 and the I plasmid JR66A, which does not carry $T^R$. JR66A eliminated all recognizable determinants (ATSu) of R62 from all 20 of 20 exconjugants tested. Thus, the tetracycline resistance of R62 was also carried by an I plasmid. This point was confirmed by showing that the P1 transductant of R62 (R62-T1), carrying only $T^R$, eliminated JR66A (Table 3).

When the prototype plasmid of the N compatibility group, N3T, was introduced into a culture carrying a tetracycline-sensitive segregant of R62, there was no exclusion of R62 and loss of the resident from only 1 in 10 exconjugants. Each plasmid was stable in and separately transferable from the doubles. Thus, the two plasmids were compatible. However, when R62 was transferred to a cell line carrying the N plasmid R390-12, the resident plasmid was eliminated from 18 of 20 exconjugants tested. Of the two exceptions, one carried both plasmids as separate entities (a stable double), proving that they were compatible. The other exception carried a recombinant plasmid conferring resistance to STC(chloramphenicol)Su (i.e., lacking resistance to ampicillin, although both parental plasmids conferred this resistance). There was no exclusion when R62 was transferred to a culture carrying the N plasmid R447b, but the resident plasmid was lost from all (20 of 20) exconjugants tested. In the reciprocal mating, there was no exclusion of R447b nor elimination of the R62 resident, but there was extensive interaction and recombination between the two (Table 4). The majority of exconjugant clones were resistant to TK (kanamycin) and transferred these markers simultaneously. These recombinants had N compatibility. Another exconjugant clone, resistant to ATK, transferred either AK resistance (presumably unmodified R447b), T resistance (an I$\alpha$ plasmid derived from R62), or ATK resistance (presumably both plasmids). Hence, R62 and N plasmids show a complex pattern of interaction. No other significant interactions between R62 and other R-factor compatibility groups were observed.

**Molecular nature of R62.** Figure 1 shows the sedimentation of the [$^3$H]thymine-labeled DNA remaining in the supernatant fluid of a cleared lysate prepared from J5-R62. Two clearly defined peaks were evident, corresponding to 83*S* and 55*S*. In some, but not all, lysates a smaller peak corresponding to approximately 47*S* was also observed on several occasions. Figure 2 shows the sedimentation properties of R62 DNA before and after treatment with deoxyribonuclease sufficient, on the average, to produce a single "nick" per molecule (1). There was a major transition of the form sedimenting at 83*S* to the 55*S* form. To a much lesser extent, there was an additional transition to a 47*S* sedimenting form. The results were consistent with transition of an 83*S* covalently closed circular (CCC) molecule to an 55*S* open circular (OC) and 47*S* linear duplex form of a single $80 \times 10^6$-dalton molecule. The inference that only a single type of CCC molecule was present was confirmed by examining the sedimentation of the DNA in alkaline sucrose gradients. Under conditions of high pH (12.5), CCC molecules form sediment at a rate approximately 3.6 times greater than that of corresponding linear or OC molecules. Only a single rapidly sedimenting DNA species with an $S_{alk}$ of 262, equivalent to $80 \times 10^6$ daltons, was observed (Fig. 3). It appeared, therefore, that the genetic traits of R62 resided on a single plasmid species. In a similar vein, analysis of the tetracycline-sensitive and streptomycin segregants of R62 showed them to be single plasmid species with molecular weights of $78 \times 10^6$ and $77 \times 10^6$,

TABLE 3. *Incompatibility of R62 and the transductant R62 (I) with group I plasmids*[a]

| Donor | Recipient | Frequency of transfer | Transconjugants tested for presence of each plasmid |
|---|---|---|---|
| J53 (R144) | J62 | $2 \times 10^{-3}$ | |
| J53 (R144) | J62 (R62) | $10^{-4}$ | 20/20 elimination of resident |
| J53 (R144) | J62 (R62-1) | $10^{-4}$ | 20/20 elimination of resident |
| J62 (R62) | J53 | $3 \times 10^{-3}$ | |
| J62 (R62) | J53 (R144) | $3 \times 10^{-6}$ | 36/36 elimination of resident or unstable doubles |
| J62 (R62) | J53 (JR66a) | $10^{-3}$ | 20/20 elimination of resident |
| J53 (JR66a) | J62 (R62) | $10^{-3}$ | 20/20 elimination of resident or unstable doubles |
| J62 (R62-T1) | J53 | $2 \times 10^{-3}$ | |
| J62 (R62-T1) | J53 (JR66a) | $10^{-4}$ | 30/30 elimination of resident |

[a] R62-T1 was the tetracycline-resistant, I-piliated P1 transductant of R62.

TABLE 4. *Interactions of R62 with plasmids of compatibility group N*

| Donor | Recipient | Frequency of transfer | Plasmids identified in transconjugants |
|---|---|---|---|
| J53 (N3T) | J62 | $5 \times 10^{-5}$ | |
| J53 (N3T) | J62 (R62-1) | $5 \times 10^{-5}$ | 11/12 both plasmids[a]<br>1/12 N3T only |
| J62 (R62-T1) | J53 | $2 \times 10^{-3}$ | |
| J62 (R62-T1) | J53 (R15) | $10^{-3}$ | 20/20 both plasmids[a] |
| J53 (R62) | J62 | $2 \times 10^{-2}$ | |
| J53 (R62) | J62 (R390-12) | $10^{-2}$ | 18/20 R62 only<br>1/20 both plasmids[a]<br>1/20 recombinant plasmid[b] |
| J53 (R62) | J62 (R447b) | $3 \times 10^{-2}$ | 20/20 R62 only |
| J62 (R447b) | J53 | $2 \times 10^{-2}$ | |
| J62 (447b) | J53 (R62) | $2 \times 10^{-2}$ | 6/10 recombinant plasmids[b]<br>2/10 unstable doubles<br>2/10 stable doubles[c] |

[a] Both plasmids stable inherited and separately transmissible.

[b] Recombinants had some, but not all, of the resistance genes of each parent. On further testing, every recombinant manifested either group I or group N compatibility.

[c] Stable doubles, but with a reassortment of resistance genes.

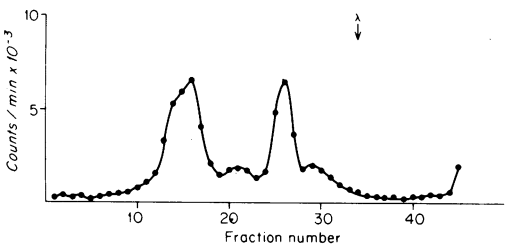


FIG. 1. *Sedimentation of R62 DNA in neutral sucrose gradient. Cleared lysate (100 μliters) of [³H]thymine-labeled E. coli R62 DNA was diluted 1:1 in TES and layerd onto a linear 5 to 20% neutral sucrose gradient. Centrifugation was carried out at 100,000 × g for 60 min at 15 C in the SW50.1 rotor of a Beckman model L3-50 preparative ultracentrifuge. Five-drop fractions were collected from a hole punctured in the bottom of the tube. Each fraction was precipitated with trichloroacetic acid, and the precipitate was collected on membrane filters and counted. Linear monomers of ¹⁴C-labeled lambda-DNA was added as a sedimentation reference (34S). The position of the lambda-DNA peak is indicated by an arrow.*

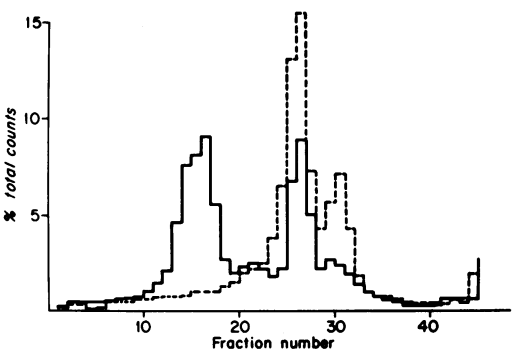


FIG. 2. *Effect of deoxyribonuclease degradation of R62 DNA. A cleared lysate of [³H]thymine-labeled R62 DNA was prepared as described in the legend to Fig. 1. The sample was divided into two parts. One sample was untreated, and the other was treated with deoxyribonuclease as described by Bazaral and Helinski (1). The samples were layered onto 5 to 20% neutral sucrose gradients and analyzed as described in the legend to Fig. 1. R62, –; R62 after DNase treatment calculated to give on the average 1 "nick" per molecule, .....*

respectively. Presumably, these segregants had suffered a small deletion of genetic material accompanying the loss of drug resistance.

Material from cleared lysates of R62 was pooled and subjected to cesium chloride-ethidium bromide equilibrium centrifugation. A dense peak, characteristic of CCC DNA, was isolated and, after extraction of the ethidium bromide and exhaustive dialysis, was examined in the electron microscope. The majority of DNA molecules present as measurable OC

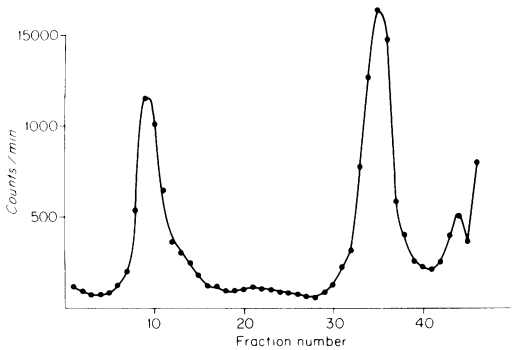


FIG. 3. *Alkaline sucrose gradient centrifugation of R62 DNA. A cleared lysate of [$^3$H]thymidine-labeled R62 DNA was layered onto a 5 to 20% alkaline (pH 12.5) sucrose gradient. The sample was centrifuged at 100,000 × g for 20 min at 20 C in the SW50.1 rotor of an L2-50 preparative ultracentrifuge. The fractions were collected and analyzed as described in the legend to Fig. 1.*

forms possessed a contour length of 38.5 ± 0.2 μm. The molecular weight of such a molecule (calculated on the assumption of 1 μm of DNA = 2.07 × $10^6$ daltons; ref. 3) was 79.7 × $10^6$, which was in excellent agreement with the molecular weight estimated from the sedimentation properties of the CCC DNA. We have, however, also consistently observed an occasional circular molecule in the electron microscope which possessed a contour length of 11.3 ± 0.1 μm, corresponding to a molecular weight of 23 × $10^6$ daltons. Although it was difficult to precisely estimate the proportion of molecules within a given preparation that were present as the 11.3-μm form, our best estimate was that this molecular type did not exceed 5% of the total plasmid DNA. That the 11.3-μm species was a minor component also seemed consistent with the results observed in sucrose gradients. A CCC molecular species equivalent to 23.3 × $10^6$ daltons is expected to possess an $S_{alk}$ value of approximately 163S. Such a species was never detected as a distinct peak in any of the lysates of J5-R62 analyzed on alkaline sucrose gradients, even though reconstruction studies indicated that such a molecule would have been readily detected by our experimental procedures if it were present in significant quantities. The amount of the 80 × $10^6$-dalton plasmid DNA identified as R62 which could be isolated from cells was equivalent to one to two copies per cell, i.e., about 6% of the total cellular DNA. Assuming our maximal estimate that 5% of the total plasmid DNA was present as the minority 11.3-μm component, it would indicate that there was less than one copy of this molecular species per cell.

The *E. coli* J5-R62 strain was cloned repeatedly with and without drug selection, and the transfer of the R62 drug-resistance determinants was carried out four times in rapid succession with selection for single and multiple drug-resistance traits. In every one of the 12 clones that were examined, only the 80 × $10^6$-dalton molecular species could be recognized in both neutral and alkaline sucrose gradients. Nonetheless, electron microscopy of the CCC molecules of several of these clones continued to reveal the small proportion (still less than one copy per cell) of the 11.3-μm molecular species. Although the estimation of the number of copies of R62 and the 11.3-μm plasmid per cell cannot be regarded as being very accurate, it seems clear that the 11.3-μm species was a minor subfraction.

**Polynucleotide sequence relationships of R62 plasmid DNA.** Covalently closed circular [$^3$H]thymine-labeled DNA samples isolated by cesium chloride-ethidium bromide centrifugation of Brij lysates of *E. coli* J5-R62 were pooled and divided into two parts. One part, containing all of the isolated CCC DNA (including the small amount of the 11.3-μm species), was used as described in Materials and Methods to determine the degree of polynucleotide sequences shared by this material with a number of unlabeled DNA preparations of well characterized plasmids. The second sample of CCC DNA was centrifuged on a neutral sucrose gradient, and only the material sedimenting in the 83S fraction (CCC molecules of 80 × $10^6$ daltons) was isolated and used in similar DNA-DNA duplex studies. As shown in Table 5, the results with both DNA samples were essentially identical. These results again confirmed that the 11.3-μm species was but a minor component of the total CCC population, and further showed that the polynucleotide sequence relationships which were recognized resided on the 80 × $10^6$-dalton molecule.

In light of the genetic studies described earlier, we were not surprised to find that approximately 57% of the R62 sequences (equivalent to roughly 47 × $10^6$ daltons) were related to the classical ColI factor and the I-class R-plasmid prototype, R144. In a reciprocal reaction, we observed that about 80% of [$^3$H]thymine-labeled R144 plasmid DNA (62 × $10^6$ daltons in size) formed DNA-DNA duplexes with unlabeled R62 DNA. An unexpected finding was that R62 shared about one-quarter of its sequences (equivalent to about 20 × $10^6$ daltons)

TABLE 5. *Relationship of R-62 to other plasmids: Direct reassociation with [³H]R62 DNA*[a]

| Plasmid | Mol wt | Compatibility group | Relative polynucleotide sequence in common (%) | | Molecular equivalent |
|---|---|---|---|---|---|
| | | | Total plasmid DNA | 83S fraction | |
| R62 | $80 \times 10^6$ | Iα | 100 | 100 | $80 \times 10^6$ |
| ColI | $65 \times 10^6$ | Iα | 54.7 | | |
| R144 | $62 \times 10^6$ | Iα | 57.0 | 58.1 | $45 \times 10^6$ |
| R1*drd*19 | $62 \times 10^6$ | FII | 8.5 | 6.2 | $6.8 \times 10^6$ |
| RTF | $50 \times 10^6$ | FII | 3.4 | | |
| $F^+$ | $62 \times 10^6$ | FI | 2.6 | | |
| N3 | $33 \times 10^6$ | N | 23.2 | 25.1 | $18.5 \times 10^6$ |
| RP4 | $35 \times 10^6$ | P | 0.3 | 0.2 | |

*Reciprocal reactions*

| Plasmid | Relative polynucleotide sequence in common (%) | Molecular equivalent |
|---|---|---|
| [³H]R144 × R62 | 80.2 | $49 \times 10^6$ |
| [³H]R1*drd* × R62 | 8.0 | $5.2 \times 10^6$ |
| [³H]N3 × R62 | 68.2 | $21.8 \times 10^6$ |

[a] [³H]R62 DNA and other labeled plasmid DNA samples were isolated by cesium chloride-ethidium bromide density equilibrium centrifugation. The R62 DNA was divided into two parts. One part (total plasmid DNA) was used without further fractionation. The other part was centrifuged on a neutral sucrose gradient, and only the material sedimenting at 83S was pooled and used (83S fraction). Each preparation of plasmid DNA was sheared and denatured, and .01 μg was reassociated in 0.14 M phosphate at 75 C with 300 μg of sheared, denatured *E. coli* DNA harboring the indicated plasmid. The degree of DNA-DNA duplex formation was assayed on hydroxylapatite as described previously (13). In every case, the degree of duplex formation was corrected for the reaction obtained with *E. coli* $F^-$ DNA, and the results were calculated relative to the homologous reaction. The actual duplex formation in the R62 homologous reaction was 90.7 (total plasmid) and 92.2% (83S fraction), with an $F^-$ contribution of 7.1% in both cases. For the reciprocal reaction, [³H]R144, [³H]R1*drd*19, and [³H]N3 DNA were reacted with their homologous DNA, *E. coli* R62 DNA and *E. coli* $F^-$ DNA. The actual extent of binding for the homologous reactions was 87, 87, and 90% for N3, R144, and R1*drd*19, respectively, corrected for an $F^-$ contribution of 7.9, 9.8, and 13.2%, respectively. The molecular equivalents were calculated as if the proportion of the total plasmid DNA forming DNA-DNA duplexes were a single contiguous length of DNA.

with the R plasmid, N3, of the N compatibility group. In the reciprocal reaction between labeled N3 DNA and unlabeled R62 DNA, we observed that some 68% of the $33 \times 10^6$-dalton N3 R plasmid DNA formed DNA-DNA duplexes with R62 DNA.

R62 DNA did not show any significant degree of DNA-DNA duplex formation with any of the representatives of other R compatibility groups that were tested. In this latter context, it is interesting to note that although R62 is phenotypically $fi^+$, it shared only 2 to 3% of its sequences (equivalent to about $2 \times 10^6$ daltons) with the classical F factor and the transfer replicon, RTF, of the FII plasmid R1*drd*19.

## DISCUSSION

The genetic and molecular findings of this investigation are consistent with the view that all of the drug-resistance, colicinogeny, $fi^+$, and I-pilus determinants of R62 reside on a single plasmid species. The elimination of ampicillin, streptomycin, spectinomycin, and sulfonamide resistance by the introduction of R144 (an Iα plasmid) is consistent with the presence of these determinants on a single physical structure (also of Iα compatibility). Cotransduction suggests close linkage between the tetracycline-resistance, colicinogeny, and I-pilus genes; the incompatibility of the $T^R$ determinant of R62 with JR66A confirms that this drug-resistance determinant is borne by an Iα plasmid. R62 excluded and was excluded by R144 less efficiently than is typical of Iα plasmids. Unlike any other two Iα plasmids, R62 and R144 formed unstable "doubles" from which recombinant R factors were obtained. Preliminary examination of these recombinants revealed that they were considerably smaller in molecular size than the sum of the two parental types and are, therefore, analogous to fused F-prime factors (3).

In its genetic interactions with plasmids of group N, R62 showed a complex pattern. In several instances, R62 and an N-class plasmid coexisted stably. From these doubles, the two plasmids were transferred separately, in unaltered form. This proves that R62 is not a member of group N. Yet, the isolation of a significant number of recombinant plasmid types from cells harboring R62 and either R390-12 or R447b indicated that there was a significant degree of genetic homology between these plasmids. It must be emphasized, however, that in the majority of cases the introduction of R62 into cell lines carrying R390-12 or R447b resulted in the loss of all recognizable resident markers. Since R62 is compatible with N plasmids, the loss of the resident plasmid in these instances presumably occurs by plasmid dislodgment (5). This phenomenon is the elimination of a resident R factor by introduction of a compatible plasmid. Although the precise mechanism of this phenomenon is unknown, we suggest that even though two plasmids may be mutually compatible during vegetative replication, during conjugal transfer one plasmid may express a gene (or genes) whose product either destroys the other or inhibits its replication.

Although it may not be an absolute prerequisite, it seems likely that dislodgment of a resident results from the introduction of a related plasmid (even though of a different compatibility group), and that the dislodgment phenomenon has some degree of specificity. In the latter context, it is interesting to note that the introduction of R447b into a culture carrying R62 was accomplished frequently by the elimination from R62 of the A, S, Su determinants. These are the precise resistance genes which are not typical of I plasmids but are common in N plasmids.

The molecular findings indicate that R62$^+$ cultures carried a single species of plasmid DNA of 80 $\times$ $10^6$ daltons. DNA-DNA duplex studies indicate that some 57% of the molecule is composed of typical I-like polynucleotide sequences, and some 25% is composed of typical N-like polynucleotide sequences. We infer that the I-like sequences are concerned with pilus production, colicin biosynthesis, and the incompatibility properties of R62. In view of the close phenotypic and genotypic similarity noted above of the drug-resistance determinants of R62 with those commonly borne on N plasmids, it is likely that some of the N-like sequences of R62 represent a drug-resistance region of the molecule. Of course, the N3 R plasmid with which the DNA-DNA duplex studies were performed does not possess the precise resistance phenotype of R62. One need not expect, however, that most of the N-like sequences of R62 are associated with drug resistance. In virtually all R plasmids studied, the drug resistance genes per se comprise but a small proportion of the plasmid and, indeed, the genetic informational content of most R plasmids remains unknown. In the present case, the genetic data suggest that even though a significant proportion of R62 is N-like, neither the incompatibility nor conjugation genes of the N plasmid are present or, if present, are not expressed. The *fi*$^+$ character of R62 has been shown to be determined by a mechanism quite different from that of F-like plasmids (24), and it is therefore not surprising that R62 shows no genetic interaction (other than fertility inhibition) nor significant DNA homology with F-like plasmids.

The results of this investigation indicate that R62 is a naturally occurring recombinant plasmid that presumably evolved as a product of an illegitimate recombinational event between an I-like and N-like plasmids. The conclusion that the recombinational event was illegitimate is based on the observation that N3 and R144, the prototype N- and I-group plasmids, respectively, share less than 2% DNA sequences in common. Moreover, typical plasmids of the I group share polynucleotide sequences only with other I-group members and with no other extrachromosomal element that we have examined. Nor have we observed the N3 plasmid to be related to any other I-group plasmid or, for that matter, to plasmids of any other R compatibility group. R62 is an exception to the rule, and it remains to be seen whether plasmids containing sequences from two such readily identifiable diverse sources are common in natural isolates. Certainly, that R plasmids can be formed by covalent linkage of replicons that separately carry resistance or transfer functions (plasmid co-integrate) is well established (3). In some cases, plasmid co-integrates may dissociate into component transfer and resistant replicons (3, 6, 12). In this respect, it is of some interest to re-examine the question of the minority 23.3 $\times$ $10^6$-dalton species observed in CCC preparations of R62. In light of our estimate that the N-like sequences of R62 are about 20 $\times$ $10^6$ daltons, it seems a reasonable hypothesis that the occasional 11.3-$\mu$m circular species may represent a dissociated N-like replicon from the ordinary single-molecular R62 plasmid co-integrate. We are currently attempting to find conditions under which sufficient quantities of the 11.3-$\mu$m species could be isolated to attempt transformation or heteroduplex studies, or both, to verify our hypothesis. Whatever may prove to be the nature of the 11.3-$\mu$m molecular species, we feel that the data presented in this communication are consistent with the view that R62 is a single genetic and physical structure which represents a naturally occurring hybrid between at least two plasmids derived from quite distinct recognizable origins. In large measure, the difference between R62 and other R plasmids previously studied is merely that the origins of the R62 plasmid co-integrate are slightly better defined.

## LITERATURE CITED

1. Bazaral, M., and D. R. Helinski. 1968. Characterization of multiple circular DNA forms of colicinogenic factor E1 from *Proteus mirabilis*. Biochemistry **7**:3513-3520.
2. Clewell, D. B., and D. R. Helinski. 1969. Supercoiled circular DNA-protein complex in *Escherichia coli*: purification and induced conversion to an open circular form. Proc. Nat. Acad. Sci. U.S.A. **62**:1159-1166.
3. Clowes, R. C. 1972. Molecular structure of bacterial plasmids. Bacteriol. Rev. **36**:361-405.
4. Clowes, R. C., and W. Hayes. 1968. Experiments in microbial genetics. Blackwell Scientific Publications, Oxford.
5. Coetzee, J. N., N. Datta, and R. W. Hedges. 1972. R factors from *Proteus rettgeri*. J. Gen. Microbiol. **72**:543-552.

6. Cohen, S. N., and C. A. Miller. 1970. Non-chromosomal antibiotic resistance in bacteria. Molecular nature of R-factors isolated from *Proteus mirabilis* and *Escherichia coli*. J. Mol. Biol. **50**:671-687.
7. Datta, N., and R. W. Hedges. 1971. Compatibility groups among *fi*⁻ R-factors. Nature (London) **234**:222-223.
8. Datta, N., and R. W. Hedges. 1973. An I-pilus determining R-factors with anomalous compatibility properties, mobilizing a gentamicin resistance plasmid. J. Gen. Microbiol. **77**:11-18.
9. Datta, N., R. W. Hedges, E. J. Shaw, R. B. Skyes, and M. H. Richmond. 1971. Properties of an R-factor from *Pseudomonas aeruginosa*. J. Bacteriol. **108**:1244-1249.
10. Datta, N., and P. Kontomichalou. 1965. Penicillinase synthesis controlled by R-factors in enterobacteriaece. Nature (London) **208**:239-241.
11. Davies, J., M. Brzezinska, and R. Benveniste. 1971. R-factors: biochemical mechanisms of resistance to aminoglycoside antibiotics. Ann. N.Y. Acad. Sci. **182**:226-233.
12. Falkow, S., L. S. Tompkins, R. P. Silver, P. Guerry, and D. J. LeBlanc. 1971. The replication of R-factor DNA in *Escherichia coli* following conjugation. Ann. N.Y. Acad. Sci. **182**:153-171.
13. Guerry, P., and S. Falkow. 1971. Polynucleotide sequence relationships among some bacterial plasmids. J. Bacteriol. **107**:372-374.
14. Hedges, R. W. 1972. Resistance to spectinomycin determined by R-factors of various compatibility groups. J. Gen. Microbiol. **72**:407-409.
15. Hedges, R. W. 1972. Phenotypic characterization of *fi*⁻ R factors determining the restriction and modification *hsp* 11 specificity. Mol. Gen. Genet. **115**:225-233.
16. Hedges, R. W., and N. Datta. 1973. Plasmids determining I pili constitute a compatibility complex. J. Gen. Microbiol. **77**:19-25.
17. Hedges, R. W., N. Datta, J. N. Coetzee, and S. Dennison. 1973. R-factors from *Proteus morganii*. J. Gen. Microbiol. **77**:249-259.
18. Hedges, R. W., N. Datta, P. Kontomichalou, and J. T. Smith. Molecular specificities of R factor-determined beta-lactamases: correlation with plasmid compatibility. J. Bacteriol. **117**:56-62.
19. Jenkins, P. G., and W. T. Drabble. 1971. β-lactamases of R factors derived from *Shigella* and *Salmonella* strains. J. Bacteriol. **108**:159-165.
20. Kleinschmidt, A. K. 1968. Monolayer techniques in electron microscopy of nucleic acid molecules, p. 361-377. *In* L. Grossman and K. Moldave (ed.) Methods in enzymology, vol. 12, part B. Academic Press Inc., New York.
21. Lawn, A. M., E. Meynell, G. G. Meynell, and N. Datta. 1967. Sex pili and the classification of sex factors in the Enterobacteriaceae. Nature (London) **216**:343-346.
22. LeBlanc, D. J., and S. Falkow. 1973. Studies on superinfection immunity among transmissible plasmids in *Escherichia coli*. J. Mol. Biol. **74**:689-701.
23. Lennox, E. S. 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology **1**:190-205.
24. Meynell, E. 1973. Pseudo-*fi*⁺ I-like sex factor, R62(I), selective for increased pilus synthesis. J. Bacteriol. **113**:502-503.
25. Meynell, G. G., and A. M. Lawn. 1968. Filamentous phages specific for I sex factor. Nature (London) **217**:1184-1186.
26. Nisioka, J., T. M. Mitani, and R. C. Clowes. 1969. Composite circular forms of R factor deoxyribonucleic acid molecules. J. Bacteriol. **97**:376-385.
27. Romero, E., and E. Meynell. 1969. Covert *fi*⁻ R factors in *fi*⁺ R⁺ strains of bacteria. J. Bacteriol. **97**:780-786.
28. Silver, R. P., and S. Falkow. 1970. Specific labeling and physical characterization of R-factor deoxyribonucleic acid in *Escherichia coli*. J. Bacteriol. **104**:331-339.
29. Watanabe, T., S. Sakaizumi, and C. Furuse. 1968. Superinfection with R factors by transduction in *Escherichia coli* and *Salmonella typhimurium*. J. Bacteriol. **96**:1796-1802.
30. Young, E. T., and R. L. Sinsheimer. 1967. Vegetative bacteriophage DNA. II. Physical characterization and replication. J. Mol. Biol. **30**:165-200.