0021-9193/78/0133-0001$02.00/0
JOURNAL OF BACTERIOLOGY, Jan. 1978, p. 1-9

Vol. 133, No. 1
Printed in U.S.A.

A ColI-Specified Product, Synthesized in Newly Infected Recipients, Limits the Amount of DNA Transferred During Conjugation of *Escherichia coli* K-12

GRAHAM J. BOULNOIS AND BRIAN M. WILKINS*

Department of Genetics, University of Leicester, Leicester, LE1 7RH, England

Received for publication 6 July 1977

The amount of ColI DNA transferred between mating cells of *Escherichia coli* K-12 increased about fourfold when rifampin-resistant donors were mated with sensitive recipients in the presence of the drug. Conjugational synthesis of ColI in *dnaB* recipients, shown primarily to reflect conversion of the transferred DNA into double-stranded material, was also enhanced when the recipients were treated with either rifampin or streptomycin. It is suggested that the amount of ColI transfer is normally limited by the synthesis of one or more proteins in the newly infected recipients. The protein is thought to be plasmid-specified because rifampin also quadrupled transfer to UV-irradiated recipients which were deficient in the transcription of the resident DNA. Successive strands of ColI appear to be transferred discontinuously, because the transferred DNA accumulated in normal and rifampin-treated recipients in the form of circular and linear monomeric units. Although rifampin treatment of recipients also increased transfer of a second Iα plasmid, R144*drd-3*, by about four times, the drug failed to cause a substantial increase of F*lac* transfer in comparable matings.

During conjugation mediated by FI and Iα plasmids (21), a specific strand of the plasmid is transferred from the donor to the recipient, where the complementary strand is synthesized (22, 24–26). This conjugational synthesis of plasmid DNA in the recipient can be measured by the incorporation of radioactively labeled thymine, provided that its uptake into both the chromosome of the recipient and the DNA of the donor is prevented (29). Incorporation of thymine into the DNA of the donor can be limited by using a strain that is deficient in thymidine kinase. Incorporation of thymine into the recipient chromosome can be prevented by using a temperature-sensitive *dnaB* mutant and mating at the restrictive temperature. Under such conditions, replication of the resident DNA ceases but the bacteria can support conjugational synthesis of plasmid DNA.

In previous experiments investigating conjugational synthesis of ColI DNA in *dnaB* recipients (29), it was observed that this synthesis was about four times greater when the activity of RNA polymerase in the recipient was inhibited by rifampin (Rif). This communication reports a detailed study of the effect of the drug on conjugational DNA synthesis and plasmid transfer.

The results suggest that the amount of ColI DNA transferred during conjugation is normally limited by the synthesis of one or more proteins in the newly infected recipients. It was found that inhibition of protein synthesis in the recipients allowed transfer of extra copies of the plasmid, thereby increasing the number of template strands available for conjugational DNA synthesis. The protein that limits transfer is thought to be specified by the incoming plasmid because its activity was detectable in UV-irradiated recipients. Such cells are deficient in transcribing the resident DNA, but they retain the ability to synthesize proteins specified by undamaged DNA (14).

The mechanism of transfer of F and Iα plasmids is shown to differ in that a comparable activity was not detected in F-mediated matings. Furthermore, in contrast to reports for F (18, 22), the transferred ColI DNA did not accumulate in the recipients in an intermediate of unusually high molecular weight, even when the amount of transfer was enhanced by rifampin treatment. Therefore, successive strands of the plasmid appear to be transferred discontinuously.

MATERIALS AND METHODS

Plasmids and bacterial strains. The properties of our stocks of Col*drd-1* and R144*drd-3* have been described previously (28, 29), and some details of pLG205 are included in Results. The F*lac* plasmid

was JCFLO (1), kindly provided by N. Willetts. The relevant properties of the bacterial strains are described in Table 1. BW67 was isolated from BW58 *polA*$^+$, selecting for a mutant capable of growing in the presence of 20 μg of uracil per ml and 10 μg of fluorodeoxyuridine per ml (5). BW69 has a deletion in the *chlA-bio* region. It was isolated as a spontaneous chlorate-resistant mutant (2), which showed pronounced UV sensitivity in subsequent screening. BW70 was obtained by co-transducing *rpsL*$^+$ into BW69, using P1*vir* grown on CSH4288 (*rpsE12*), and selecting for spectinomycin resistance.

Growth of bacteria. Bacteria were grown and mated in a salts-glucose-Casamino Acids (SGC) medium containing, per liter: Na_2HPO_4, 6 g; KH_2PO_4, 3 g; NH_4Cl, 1 g; NaCl, 0.5 g; $MgSO_4$, 0.12 g; $CaCl_2$, 11 mg; glucose, 4 g; Casamino Acids (Difco), 2 g; thiamine HCl, 1 mg. This was routinely supplemented with 0.2 mg of biotin per liter for experiments involving BW70. Overnight cultures were diluted and grown for three to four mass doublings to about 2×10^8 bacteria per ml before use in matings. Thymine-requiring strains were grown and mated in the presence of 2 μg of thymine and 200 μg of deoxyguanosine per ml (31). The *dna*$^+$ and *dnaB* strains were grown at 37 and 33°C, respectively.

Matings. Mating mixtures usually consisted of 3.5 ml, and they initially contained about 10^8 bacteria of each parental strain per ml. The mixtures were gently swirled in 150-ml Erlenmeyer flasks. When matings involved *dnaB* recipients and rifampin treatment, the recipients were first incubated at 43°C for 5 min. Rifampin (Sigma) was then added to the concentration required in the mating, and incubation was continued for a further 5 min. After this period, prewarmed donors and [^{14}C]thymine, both in rifampin-containing medium, were added to start the mating. When streptomycin sulfate (Glaxo) was used, the *dnaB* recipients were incubated at 43°C in the presence of the drug for 15 min before mating. When *uvr dna*$^+$ recipients were used, the bacteria were irradiated by using the procedure described previously (29). They were not incubated with rifampin before mating because the irradiated bacteria may lose the capacity to act as recipients if mating is delayed (29).

Thymine incorporation. [2-^{14}C]thymine and [*methyl*-^{3}H]thymine (The Radiochemical Centre, Amersham) were always conditioned before addition to matings, using the following method. The radiochemicals were added to a small volume of exponentially growing BW40 at about 10^8 bacteria per ml of SGC medium. After incubation at 37°C for about 25 min, the bacteria were removed by filtration, and the filtrate was stored on ice for up to 2 h. This procedure removed trace amounts of undefined labeled compounds that were rapidly incorporated into trichloroacetic acid-precipitable material by both *tdk* and *thyA dnaB* bacteria. Thymine incorporation was measured as described previously (29). [^{14}C]- and [^{3}H]thymine were used at 0.25 and 10 μCi/μg, respectively. The final concentration of thymine was always 2 μg/ml.

Selective lysis of donors. A 2.5-ml amount of mating mixture was added to an equal volume of ice-cold S buffer (consisting of the six salts in SGC medium) supplemented with 0.01 M KCN and 40 μg of tryptophan and 100 μg of thymine per ml. The mixture was blended for 3 s (17). The bacteria were then rapidly sedimented, resuspended in 2.5 ml of S buffer containing KCN, tryptophan, and thymine, and added to 1 ml of buffer containing 10^{12} phage T6. After 5 min at 40°C, a previous procedure (30) was used involving the addition of deoxyribonuclease and ribonuclease, followed by Pronase and then Brij 58. The T6^r recipient bacteria were then washed three times at 4°C by sedimentation and resuspension in buffer containing 5 mM KCN and 100 μg of thymine per ml. About 2×10^8 BW40 cells, UV irradiated with 500 J/m^2, were added as carrier to promote pellet formation.

Sedimentation analysis of transferred DNA. After selective lysis of the donors, the washed recipients were gently resuspended in 0.7 ml of lysis buffer at pH 9.1 (11). The yield of covalently closed circular (CCC) DNA was measured after addition of 0.1 ml of the resuspended cells to 0.05 ml of 1% sodium dodecyl sulfate in 0.8 M NaOH (10), which had been previously layered on top of an alkaline sucrose gradient. Each gradient consisted of 4.1 ml of 5 to 20% (wt/vol) sucrose in 0.7 M NaCl, 0.3 M NaOH, and 0.01 M ethylenediaminetetraacetic acid on a 0.25-ml shelf of

TABLE 1. *Bacterial strains*

Strain	Plasmid	Relevant genotype[a]	Derivation
BW40		*tdk-1 uvrB5 tsx-33 rpsL31*	(30)
BW67		*dnaB70 deoA deoC tdk tsx rpsL* Col$^{r b}$	From BW58 *polA*$^+$ (29)
BW68		*dnaB70 thyA deoB deoC tsx rpsL* Col^r	From BW56 *polA*$^+$ (29)
BW69		*dnaB70 thyA deoB deoC* Δ(*chlA-uvrB-bio*) *tsx rpsL* Col^r	From BW68
BW70		*dnaB70 thyA deoB deoC* Δ(*chlA-uvrB-bio*) *tsx rpsE12* Col^r	From BW69
BW72	Col*drd-1*	*tdk-1 uvrB5 rpsL31 rpoB* Col^r	From BW51 (29)
BW73	Col*drd-1*	*thyA deoB uvrB5 rpsL31 rpoB* Col^r	From BW50 (29)
BW74	F*lac*$^+$	*thyA deoB uvrB5 rpsL31 rpoB*	From BW50 (29)
BW76	R144*drd-3*	*tdk-1 uvrB5 rpsL31 rpoB* Col^r	From BW51 (29)

[a] The genotypic symbols are defined in Bachmann et al. (4). Other markers considered to be redundant to this work can be found in the references in the last column.

[b] Phenotypic symbol for colicin I resistance. It is not known whether the mutation is in *cir* or *tonB*.

70% sucrose. The gradients were centrifuged in a Beckman SW56 rotor at 20°C. Fractions (5-drop) were collected on 2.5-cm-square pieces of Whatman no. 1 filter paper, which were washed as described previously (26). The more slowly sedimenting DNA was analyzed by adding 0.1 ml of cells in lysis buffer to 0.1 ml of alkaline sodium dodecyl sulfate layered on top of a 12.5-ml 5 to 20% alkaline sucrose gradient above a 0.7-ml shelf. These gradients were centrifuged in a Beckman SW40 rotor at 20°C. Fractions (10-drop) were collected on 2.5-cm-diameter circles of Whatman no. 3 filter paper. Marker plasmid DNA was isolated as CCC DNA from an appropriate strain. The cells were lysed, using lysozyme and Sarkosyl, and CCC DNA was isolated by equilibrium centrifugation in CsCl-ethidium bromide (26).

RESULTS

Effect of rifampin on conjugational DNA synthesis. ColI*drd-1* donors, which were defective in thymine incorporation due to a mutation at *tdk*, were mated with thymine-requiring *dnaB* recipients at 43°C to measure conjugational synthesis of plasmid DNA in the recipients. Rifampin-resistant donors, mutant at *rpoB*, were used in these experiments to minimize the effects of the drug on the expression of functions required for plasmid transfer. Figure 1 shows that addition of rifampin to the mating stimulated the amount of conjugational DNA synthesis; after 1 h, about four times more [^{14}C]thymine had been incorporated in the treated mating than in the control, in agreement with previous data (29). When the parental cultures were incubated separately in this type of experiment, they incorporated about 150 cpm/ml in 1 h. Thus about 85% of the counts incorporated at 60 min in the control and at least 95% of those in the treated mating can be attributed to the synthesis of plasmid DNA in the recipients.

The line of data at the top of Fig. 1 indicates the percentage of the recipients that had acquired at least one copy of the plasmid at different times in such a mating.

One interpretation of the enhanced conjugational DNA synthesis is that rifampin increased the amount of DNA transferred. This hypothesis was tested by adding phage T6 at a high multiplicity of infection to half of the treated mating at 30 min in order to lyse the T6^s donors. The addition of T6 abruptly stopped [^{14}C]thymine incorporation (Fig. 1). This shows that the presence of intact donors was essential for the rifampin-induced stimulation of DNA synthesis in the recipients, and it implies that the stimulation reflects increased DNA transfer. In contrast to the rifampin-treated mating, incorporation of [^{14}C]thymine continued to increase slowly after addition of T6 to the control mating.

To determine the duration of conjugational DNA synthesis in this experimental system, the basic experiment described in Fig. 1 was repeated, but for a longer mating period. Incorporation of [^{14}C]thymine ceased in both the ri-

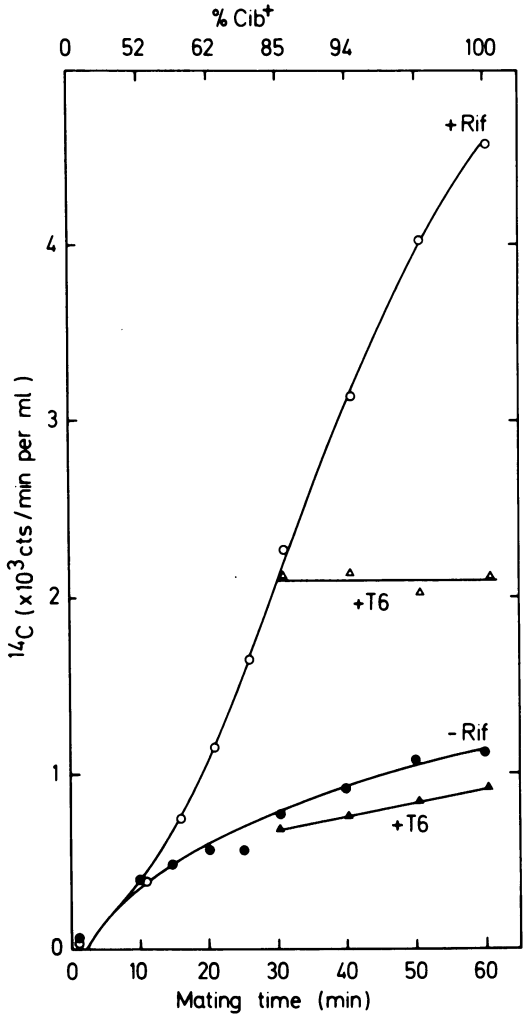

FIG. 1. *Kinetics of [^{14}C]thymine incorporation into Rif^s dnaB recipients mated with ColIdrd-1 donors in the presence* (○) *or absence* (●) *of 200 μg of rifampin per ml. BW72 was mated with BW68 at 43°C. At 30 min, 2-ml volumes of the matings were diluted into 2 ml of SGC containing [^{14}C]thymine and either 4.3 × 10^{11} T6 phage particles, UV irradiated with 120 J/m^2* (△, *+Rif;* ▲, *−Rif*), *or T2 adsorption buffer* (○, *+Rif;* ●, *−Rif*). *The counts incorporated after 30 min have been doubled to allow for the dilution. The % Cib$^+$ values indicate the percentage of recipients that had acquired the plasmid at 0, 10, 20, 30, 40, and 60 min in such a mating. These values were obtained in an interrupted-mating experiment. After interruption, the donors were eliminated by the addition of T6, and the recipients were incubated on nutrient agar at 30°C to allow formation of colonies. Each value was obtained by scoring about 350 colonies for the Cib$^+$ (colicin Ib-producing) phenotype (29).*

fampin-treated and control matings at about 100 min. The rifampin-induced stimulation of conjugational DNA synthesis at 100 min was 5.5 times.

The recipients used in these experiments were Rif^s, whereas the donors were Rif^r. These properties suggest that the drug enhanced conjugational DNA synthesis by inhibiting the action of RNA polymerase in the recipients. In confirmation, it was found that rifampin had little effect on conjugational DNA synthesis if both parents were Rif^r. When a Rif^r mutant of BW68 was mated with BW72, the amounts of [^{14}C]-thymine incorporated in 60 min in the control and treated matings were 869 and 1,133 cpm/ml, respectively.

Effect of rifampin on DNA transfer. The effect of rifampin on DNA transfer was measured directly by labeling the DNA of thymine-requiring Rif^r donors and mating them with *tdk* Rif^s recipients. After 60 min, the $T6^s$ donors were lysed by the addition of phage T6, and the exposed DNA was removed by treatment with deoxyribonuclease and washing. The amount of radioactivity remaining after the selective lysis of the donors reflects the amount of DNA transferred to the $T6^r$ recipients. The presence of the *dnaB70* allele in the recipients was inessential in this experiment, but it was included to make the strain as isogenic as possible with the recipients described in Fig. 1.

The total amount of DNA transferred was measured with donors that were grown both before and during mating in the presence of [^{14}C]thymine, and it is shown in Table 2 (lines 1–3) in the form of radioactivity resisting T6 lysis. Addition of rifampin to the mating mixture was found to quadruple the total amount of DNA transfer, the data being in good agreement with the amounts of conjugational DNA synthesis shown in Fig. 1.

To determine whether rifampin enhances the transfer of preexisting plasmids or DNA made during the mating, the experiment was repeated with donors that were grown in [^{14}C]thymine either before or during mating. Although rifampin doubled the amount of preexisting DNA that was transferred (Table 2, lines 4 and 5), it enhanced the transfer of DNA that was made during the mating by about eight times (Table 2, lines 6 and 7). Thus, the drug primarily affects transfer by promoting the transmission of DNA made during the mating period.

Effect of streptomycin on conjugational DNA synthesis. Rifampin may enhance ColI DNA transfer by inhibiting the synthesis of RNA required for protein synthesis. This possibility was examined by determining the effect of streptomycin (Str) on conjugational DNA synthesis in matings of Str^r donors and Str^s recipients. The recipient strain was descended from the same strain that was used in the matings involving rifampin. The results in Fig. 2 show that the presence of 100 μg of streptomycin per ml doubled the amount of conjugational DNA synthesis in a 60-min mating, but 50 or 30 μg/ml had a progressively greater stimulatory effect. The recipients were incubated in the presence of the drug for 15 min before mating, because the lowest concentration of streptomycin used gradually inhibited protein synthesis over about 30 min. When BW70 was incubated at 33°C in

TABLE 2. *Effect of rifampin on the transfer of ColI to dnaB recipients*[a]

Labeling procedure	Rifampin (μg/ml)	No. of expt	cpm/ml		
			Mating (M)	Control (C)	Transfer (M − C)
Before and during mating	0	2	1,169	8	1,161
	120	1	4,484	100	4,384
	200	2	4,585	2	4,583
Before mating[b]	0	2	738	2	736
	200	2	1,461	0	1,461
During mating[c]	0	1	347	30	317
	120	1	2,542	30	2,512

[a] BW73 was mated with BW67 for 1 h at 43°C. The amount of trichloroacetic acid-precipitable radioactivity was determined after selective lysis of the donors. The controls consisted of the parental cultures incubated separately for 1 h and then mixed immediately before lysis of the donors.

[b] The donors were incubated in the presence of [^{14}C]thymine for three to four mass doublings. Immediately before mating, they were sedimented and then resuspended and mated in SGC containing 50 μg of nonradioactive thymine per ml.

[c] The donors were grown before mating in the presence of nonradioactive thymine. [^{14}C]thymine was added to the mating mixture.

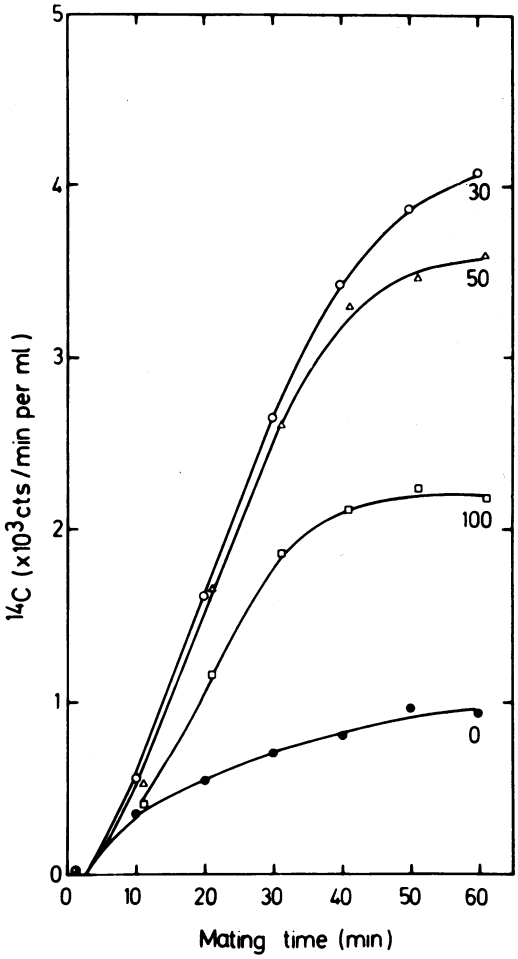

FIG. 2. *Kinetics of [^{14}C]thymine incorporation into Str^s dnaB recipients mated with ColIdrd-1 donors in the presence of various concentrations of streptomycin. BW72 was mated with BW70 at 43°C. The concentrations of streptomycin (micrograms per milliliter) were: ●, 0, ○, 30; △, 50; □, 100.*

30 μg of streptomycin per ml, [^{14}C]leucine incorporation into trichloroacetic acid-precipitable material in the period equivalent to mating (15 to 75 min after the addition of the drug) was 9% of the value for the untreated control.

Comparison of the data in Fig. 1 and 2 shows that the stimulatory effect of rifampin on conjugational DNA synthesis can be reproduced using 30 μg of streptomycin per ml. This implies that both drugs enhance DNA transfer by inhibiting the synthesis of one or more proteins in the recipient.

Inhibition of transcription of the chromosome in the recipients. Bacteria, defective in DNA repair by the excision process (*uvr*) and extensively irradiated with UV, retain the ability to act as recipients in mating (9, 12, 29). The damaged DNA is an unsuitable template for RNA synthesis. For example, exposure of BW69 to 400 J of UV per m^2 reduced [^{14}C]uracil incorporation into trichloroacetic acid-precipitable material in a 2-min pulse to about 1% of the unirradiated control value. Such irradiated cells can still support the synthesis of proteins specified by undamaged plasmid or phage DNA introduced after irradiation (9, 14). Use of irradiated recipients therefore provides a system for determining whether rifampin treatment enhances transfer by affecting the expression of plasmid rather than bacterial genes; if the drug causes increased transfer by inhibiting transcription of the immigrant plasmid, it should still enhance transfer to irradiated cells.

Table 3 shows that rifampin enhanced DNA transfer by more than four times when ColI*drd-1* donors were mated for 60 min with excision-defective recipients, UV irradiated with 400 J/m^2. The matings were incubated at 43 and 37°C to ensure that the effect of the drug was temperature independent. Thus these results, showing enhanced transfer to rifampin-treated, irradiated recipients, imply that inhibition of synthesis of plasmid-specified products in newly infected recipients allows plasmid transfer to continue.

Effect of rifampin on F*lac* transfer. Inhibition of gene expression in newly formed transconjugants may allow increased transfer of other types of conjugative plasmid. This was investigated by determining the effect of rifampin on F*lac* transfer to irradiated Rif^s recipients. The results (Table 3) show that the drug increased

TABLE 3. *Effect of rifampin on plasmid transfer to UV-irradiated dna$^+$ Rif^s recipients*[a]

Plasmid	Temp (°C)	cpm transferred/ml		+Rif/−Rif
		−Rif	+Rif	
ColI*drd-1*[b]	43	924	4,134	4.47
	37	518	2,270	4.38
F*lac*[c]	37	456	542	1.19

[a] In all matings the recipients were UV irradiated with 400 J/m^2, and the DNA in the donors was labeled before and during mating with [^{14}C]thymine. Counts transferred were measured after selective lysis of the donors. The data have been corrected to allow for the radioactivity resisting T6 lysis in the control experiments (about 60 cpm/ml). In these controls, the parental cultures were incubated separately for 1 h and were then mixed immediately before selective lysis of the donors.

[b] BW73 was mated with a colicin-resistant mutant of BW40 for 1 h at either 43 or 37°C. Each line contains the values derived in a single experiment. Rifampin was added to give 200 μg/ml.

[c] BW74 was mated with BW40 for 1 h at 37°C. The data are the average of five separate experiments. Rifampin was added to give 140 μg/ml.

transfer, as measured by the amount of radioactivity resistant to T6 lysis, by about 20%. The values obtained in the five experiments ranged from a reduction in transfer of 12% to an increase of 41%. These results show that rifampin treatment of recipients has little or no effect on F*lac* transfer, in marked contrast to matings involving ColI.

The concentration of rifampin used is considered to be suitable for maximizing any effect of the drug on F*lac* transfer. This was determined with the technically simpler system of measuring conjugational DNA synthesis in irradiated *dna*$^+$ recipients (29). The slight rifampin-induced increment of synthesis detected in such matings (29) was not significantly altered by decreasing the concentration of the drug from 140 to 35 μg/ml (unpublished data).

UV irradiation of BW40 recipients curtails their capacity to receive F*lac*, a dose of 400 J/m^2 reducing by about 50% the amount of DNA transferred in a 60-min mating (B. M. Wilkins and S. E. Hollom, unpublished data). Thus a potential rifampin-induced increase of F*lac* transfer may never develop if the recipients are irradiated. To examine this possibility, the F*lac* matings described in Table 3 were repeated with unirradiated BW40 as recipients. Rifampin treatment of these undamaged bacteria again failed to cause a marked increase of F*lac* transfer; the average number of counts per minute transferred per milliliter in two separate experiments was 1,046 and 1,589 for the control and treated matings, respectively.

Effect of rifampin on matings mediated by a second Iα plasmid. Although rifampin treatment of recipients quadrupled transfer of ColI*drd-1*, it only slightly increased the transmission of F*lac*. However, the phenomenon of enhanced transfer is not restricted to ColI-mediated matings because a 3.9-fold increase of conjugational DNA synthesis was detected in rifampin-treated *dnaB* recipients mated with donors of another Iα plasmid, R144*drd-3*. When BW76 was mated with BW68 in three experiments similar to that described in Fig. 1, the average amount of [^{14}C]thymine incorporated in the untreated *dnaB* recipients was 1,509 cpm/ml at 60 min. An average of 5,856 cpm/ml was incorporated into the rifampin-treated recipients.

Sedimentation properties of the transferred DNA. The sedimentation properties of the transferred ColI DNA were examined in alkaline sucrose gradients to determine whether the increased transfer to rifampin-treated recipients was associated with the appearance of novel species of plasmid DNA. Thymine-requiring ColI*drd-1* donors were labeled before and during mating with *tdk dnaB* recipients. After mating, the donors were selectively lysed with phage T6. The washed recipients were then lysed in alkaline sodium dodecyl sulfate on top of alkaline sucrose gradients. pLG205 DNA was added to the gradients as a marker to indicate the expected sedimentation position of dimeric molecules. pLG205 is a ColIb-prime plasmid carrying *E. coli* K-12 DNA. Its molecular weight, as judged by sedimentation in neutral sucrose gradients, is about 1.2 × 10^8 (M. I. Sedgwick, unpublished data). This is approximately twice the molecular weight of ColIb, which is reported as 6.2 × 10^7 (7; Sedgwick, unpublished data).

The transferred DNA from the untreated mating was found in two approximately equal peaks when centrifuged for a short period (Fig. 3). The rapidly sedimenting material can be identified from the sedimentation pattern of the markers as CCC monomeric units. The majority of the more slowly sedimenting material cosed-

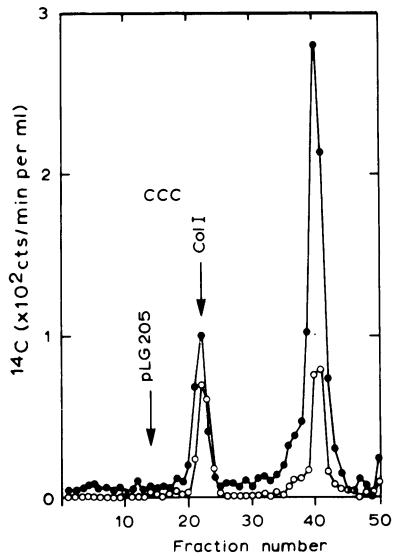

FIG. 3. *Sedimentation analysis of transferred ColI DNA to detect the presence of CCC molecules. BW73 was mated with BW67 for 1 h at 43° C in the presence (●) or absence (○) of 200 μg of rifampin per ml. The DNA in the donors was labeled with [^{14}C]thymine both before and during the mating. After selective lysis of the donors, the washed recipients were lysed in alkaline sodium dodecyl sulfate (SDS) on top of 4.1-ml alkaline sucrose gradients. The gradients were centrifuged for 37 min at 30,000 rpm. The direction of sedimentation was from right to left. The arrows refer to the fractions containing peaks of rapidly sedimenting, labeled DNA when ColI and pLG205 DNA, isolated as CCC material, were sedimented accordingly. Sedimentation rates cannot be calculated from these gradients because the SDS on the gradients reduced the volume of the drops corresponding to the top 10 fractions.*

imented with monomeric single strands of ColI marker.

The transferred DNA isolated from the rifampin-treated recipients gave the same two peaks, but the fraction of label in CCC DNA was less than in the control. The difference in the relative amounts of CCC DNA is attributed to experimental variation. The difference does not reflect inability of the recipients to circularize the extra transferred DNA in rifampin-treated matings, because when the experiment was repeated with donors labeled solely during mating, the size of the two peaks was equal. These results therefore show that rifampin does not inhibit the conversion of the transferred DNA into CCC molecules. Furthermore, the drug does not promote the formation of detectable amounts of covalently closed dimeric circles.

The homogeneity of the more slowly sedimenting transferred DNA was investigated by longer centrifugation of the lysates (Fig. 4). The radioactivity at the bottom of the gradients is assumed to be in CCC DNA. The majority of the remaining labeled material was found in two peaks. These can be identified from the sedimentation positions of the marker DNA as circular and linear single-stranded monomeric units of ColI. There is little accumulation of high-molecular-weight species in the rifampin-treated recipients. Thus the processing of the incoming DNA appears to be normal in the presence of the drug.

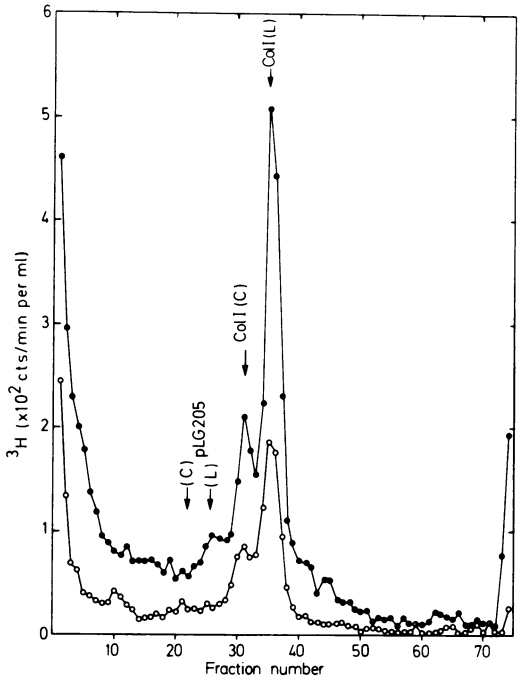

FIG. 4. *Sedimentation analysis of transferred ColI DNA to characterize the linear molecules. The procedure was the same as that for Fig. 3, except that the matings were for 45 min and 12.5-ml gradients were used. The latter were centrifuged for 3 h at 35,000 rpm. The arrows refer to the fractions containing peaks of radioactivity when ColI and pLG205 DNA were sedimented accordingly. Each reference plasmid gave two peaks, which are assumed to correspond to circular (C) and linear (L) single-stranded DNA (23).*

DISCUSSION

The conjugational synthesis of ColI DNA detected in *dnaB* recipients predominantly reflects the conversion of the transferred DNA into double-stranded material. This is indicated by the correspondence between the amount of DNA transferred from the defined donor strain (Table 2) and the extent of the synthesis in the recipients (Fig. 1). Thus, measurement of conjugational DNA synthesis can be used as a relatively simple, but indirect, method for estimating DNA transfer. The resolution of the method is limited by the degree to which the transconjugants become active donors as the mating proceeds. The relative importance of such secondary transfer will be amplified by measuring conjugational DNA synthesis, because the transconjugants, being mutant at *thyA*, will incorporate thymine into the DNA synthesized to replace the transferred strands. The slight increase in thymine incorporation after T6 lysis of the defined *tdk* donors in the control mating in Fig. 1 suggests that a small fraction of the plasmid transconjugants do become active donors in a 1-h mating.

Transcription of an F-like R plasmid is asymmetric and occurs on the same strand of DNA as that transferred during mating (27). This raises the possibility that conjugational synthesis of plasmid DNA, which differs from vegetative replication in lacking a requirement for *dnaB* product, requires plasmid-specified functions that are encoded in the transferred strand and expressed in the recipient. In the case of ColI, this possibility is ruled out by the observation that conjugational synthesis in the recipient is resistant to rifampin.

The most striking effect of rifampin on conjugational synthesis of ColI DNA is the fourfold stimulation detected when Rif^r donors were mated with Rif^s recipients (Fig. 1). This stimulation was caused by enhanced transfer from the donors, which amounted at 60 min to about seven single-stranded equivalents of ColI per recipient cell. This calculation is based on the data for conjugational DNA synthesis at 10 and 60 min in the untreated mating in Fig. 1. At 10 min, 52% of the recipients had received at least one copy of the plasmid and 340 cpm/ml had

been incorporated into DNA synthesized as a result of mating.

Streptomycin treatment of the recipients also increased conjugational synthesis of ColI DNA, presumably by enhancing the amount of transfer. The magnitude of the increase was inversely related to the streptomycin concentration (Fig. 2). The basis of this relationship is not understood. It may reflect damage to the cell membranes of the sensitive bacteria (3), resulting in loss of ability of the recipients to maintain effective unions with the donors. However, the quadrupled conjugational synthesis detected when the recipients were treated with 30 μg of streptomycin per ml clearly suggests that enhanced transfer results from the inhibition of synthesis of at least one species of protein in the recipient. In a normal untreated mating, this protein, which is synthesized in the newly infected recipients, must limit the amount of DNA transfer. The results of the experiments with UV-irradiated recipients (Table 3) imply that the protein is specified by the incoming plasmid rather than the bacterial chromosome. Specification of such a transfer-limiting function may be a general property of Iα plasmids, because rifampin treatment of *dnaB* recipients also resulted in about a fourfold increase of conjugational DNA synthesis in matings mediated by R144*drd-3*.

Elucidation of the mode of action of such a protein will probably require the isolation of transfer mutants of Iα plasmids. Because the Iα plasmids have not been well studied genetically, we examined whether or not a similar protein is active in limiting DNA transfer in F-mediated matings. The results show that rifampin treatment of recipients only slightly increased the amount of F*lac* DNA transferred, in marked contrast to ColI*drd-1* or R144*drd-3*. The present data are consistent with previous measurements of conjugational synthesis of F*lac* DNA in irradiated *dna*$^+$ recipients, which showed a 56% stimulation of synthesis in rifampin-treated recipients (29). The rifampin-induced increase of F*lac* transfer, which reached a maximum of about 52% when unirradiated recipients were used, is considered to be too small to allow exploitation of existing F mutants to analyze its nature.

A clue to the role of the ColI-specified protein is provided by the observation that rifampin treatment doubled the amount of transfer of DNA that existed in the donors before mating (Table 2). This implies that only half of the preexisting plasmids had the opportunity to transmit DNA in a normal untreated mating. The protein therefore seems to limit transfer by destroying the competence of the newly formed transconjugants to act as recipients. Its activity is detectable in a population of recipients within 10 to 15 min of mating (Fig. 1 and 2).

The mode of action of the protein is unknown. It is possibly involved in surface or entry exclusion. This property of plasmid-containing cells reduces their ability to act as recipients in matings with bacteria harboring the same or a related plasmid (20). This possibility is open to the criticism that whereas the effect of rifampin on F and ColI transfer differs, both plasmids cause efficient exclusion in established strains (19, 20). Resolution of the possibility may require the isolation of a transfer-proficient, exclusion-defective mutant of an Iα plasmid and an understanding of the mechanism and kinetics of expression of exclusion in F and Iα plasmid transconjugants.

Conversion of transferred ColI DNA into CCC monomeric molecules occurred in rifampin-treated recipients, indicating that the process is not interrupted if the synthesis of plasmid-specified products is inhibited in the newly formed transconjugants. The same conclusion has been reached for F (15). The reactions involved in circularization are not understood. The rolling-circle model (13) provides an appealing explanation because circular molecules might be generated from high-molecular-weight intermediates by recombination within the repeated regions (22). Evidence suggesting the existence of such intermediates in 40-min matings involving F has been given (18, 22). However, DNA of unusually high molecular weight did not accumulate in 45-min, ColI-mediated matings (Fig. 4), even in rifampin-treated recipients which, it is calculated from the data in Fig. 1, had received the equivalent of at least five single strands of the plasmid. Fenwick and Curtiss (10) likewise failed to detect abnormally large molecules of transferred DNA in minicells mated with donors of another Iα plasmid. It seems probable, therefore, that transfer of Iα plasmids involves the transmission of defined lengths of DNA, which are basically of monomeric length.

Two models for the circularization of essentially monomeric strands of transferred DNA have been proposed (8). Both involve a degree of terminal redundancy in the transferred strand. However, circularization of precise monomeric lengths could occur if the transferred DNA contains two pairs of inverted complementary sequences. These might allow circularization before synthesis of the complementary strand by the scheme proposed for the closure of the viral strand of single-stranded phages (Fig. 8–18 in reference 16). Alternatively, one pair of sequences might promote the synthesis of the complementary strand and the other the formation of terminal redundancy by a scheme

involving the principles of the model of Cavalier-Smith (6).

ACKNOWLEDGMENTS

We gratefully acknowledge the excellent technical help of Mary Beddoes and Ian Sedgwick's supply of ColI and pLG205 DNA for use as markers.

G.J.B. was supported by a Science Research Council Studentship.

LITERATURE CITED

1. **Achtman, M., N. Willetts, and A. J. Clark.** 1971. Beginning a genetic analysis of conjugational transfer determined by the F factor in *Escherichia coli* by isolation and characterization of transfer-deficient mutants. J. Bacteriol. **106**:529–538.
2. **Adhya, S., P. Cleary, and A. Campbell.** 1968. A deletion analysis of prophage lambda and adjacent genetic regions. Proc. Natl. Acad. Sci. U.S.A. **61**:956–962.
3. **Anand, N., and B. D. Davis.** 1960. Effect of streptomycin on *Escherichia coli*. Nature (London) **185**:22–23.
4. **Bachmann, B. J., K. B. Low, and A. L. Taylor.** 1976. Recalibrated linkage map of *Escherichia coli* K-12. Bacteriol. Rev. **40**:116–167.
5. **Beck, C. F., J. L. Ingraham, J. Neuhard, and E. Thomassen.** 1972. Metabolism of pyrimidines and pyrimidine nucleosides by *Salmonella typhimurium*. J. Bacteriol. **110**:219–228.
6. **Cavalier-Smith, T.** 1974. Palindromic base sequences and replication of eukaryote chromosome ends. Nature (London) **250**:467–470.
7. **Clewell, D. B., and D. R. Helinski.** 1970. Existence of the colicinogenic factor-sex factor *Col*I_b-P9 as a supercoiled circular DNA-protein relaxation complex. Biochem. Biophys. Res. Commun. **41**:150–156.
8. **Curtiss, R., III, and R. G. Fenwick, Jr.** 1975. Mechanism of conjugal plasmid transfer, p. 156–165. *In* D. Schlessinger (ed.), Microbiology—1974. American Society for Microbiology, Washington, D.C.
9. **Falkow, S., L. S. Tomkins, R. P. Silver, P. Guerry, and D. J. LeBlanc.** 1971. The replication of R-factor DNA in *Escherichia coli* K-12 following conjugation. Ann. N.Y. Acad. Sci. **182**:153–171.
10. **Fenwick, R. G., Jr., and R. Curtiss III.** 1973. Conjugal deoxyribonucleic acid replication by *Escherichia coli* K-12: stimulation in *dnaB*(ts) donors by minicells. J. Bacteriol. **116**:1212–1223.
11. **Freifelder, D., A. Folkmanis, and I. Kirschner.** 1971. Studies on *Escherichia coli* sex factors: evidence that covalent circles exist within cells and the general problem of isolation of covalent circles. J. Bacteriol. **105**:722–727.
12. **Freifelder, D. R., and D. Freifelder.** 1968. Studies on *Escherichia coli* sex factors. I. Specific labeling of F'Lac DNA. J. Mol. Biol. **32**:15–23.
13. **Gilbert, W., and D. Dressler.** 1968. DNA replication: the rolling circle model. Cold Spring Harbor Symp. Quant. Biol. **33**:473–484.
14. **Hendrix, R. W.** 1971. Identification of proteins coded in phage lambda, p. 355–370. *In* A. D. Hershey (ed.), The bacteriophage lambda. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
15. **Hiraga, S., and T. Saitoh.** 1975. F deoxyribonucleic acid transferred to recipient cells in the presence of rifampin. J. Bacteriol. **121**:1000–1006.
16. **Kornberg, A.** 1974. DNA synthesis. W. H. Freeman & Co., San Francisco.
17. **Low, B., and T. H. Wood.** 1965. A quick and efficient method for interruption of bacterial conjugation. Genet. Res. **6**:300–303.
18. **Matsubara, K.** 1968. Properties of sex factor and related episomes isolated from purified *Escherichia coli* zygote cells. J. Mol. Biol. **38**:89–108.
19. **Meynell, G. G.** 1969. Exclusion, superinfection immunity and abortive recombinants in $I^+ \times I^+$ bacterial crosses. Genet. Res. **13**:113–115.
20. **Novick, R. P.** 1969. Extrachromosomal inheritance in bacteria. Bacteriol. Rev. **33**:210–263.
21. **Novick, R. P., R. C. Clowes, S. N. Cohen, R. Curtiss III, N. Datta, and S. Falkow.** 1976. Uniform nomenclature for bacterial plasmids: a proposal. Bacteriol. Rev. **40**:168–189.
22. **Ohki, M., and J. Tomizawa.** 1968. Asymmetric transfer of DNA strands in bacterial conjugation. Cold Spring Harbor Symp. Quant. Biol. **33**:651–657.
23. **Rhoades, M., and C. A. Thomas, Jr.** 1968. The P22 bacteriophage DNA molecule. II. Circular intracellular forms. J. Mol. Biol. **37**:41–61.
24. **Rupp, W. D., and G. Ihler.** 1968. Strand selection during bacterial mating. Cold Spring Harbor Symp. Quant. Biol. **33**:647–650.
25. **Vapnek, D., M. B. Lipman, and W. D. Rupp.** 1971. Physical properties and mechanism of transfer of R factors in *Escherichia coli*. J. Bacteriol. **108**:508–514.
26. **Vapnek, D., and W. D. Rupp.** 1970. Asymmetric segregation of the complementary sex-factor DNA strands during conjugation in *Escherichia coli*. J. Mol. Biol. **53**:287–303.
27. **Vapnek, D., and E. Spingler.** 1974. Asymmetry and extent of in vivo transcription of R-plasmid deoxyribonucleic acid in *Escherichia coli*. J. Bacteriol. **120**:1274–1278.
28. **Wilkins, B. M.** 1975. Partial suppression of the phenotype of *Escherichia coli* K-12 *dnaG* mutants by some I-like conjugative plasmids. J. Bacteriol. **122**:899–904.
29. **Wilkins, B. M., and S. E. Hollom.** 1974. Conjugational synthesis of F*lac*$^+$ and ColI DNA in the presence of rifampicin and in *Escherichia coli* K12 mutants defective in DNA synthesis. Mol. Gen. Genet. **134**:143–156.
30. **Wilkins, B. M., S. E. Hollom, and W. D. Rupp.** 1971. Deoxyribonucleic acid transferred from ultraviolet-irradiated excision-defective Hfr cells of *Escherichia coli* K-12. J. Bacteriol. **107**:505–512.
31. **Zaritsky, A., and R. H. Pritchard.** 1971. Replication time of the chromosome in thymineless mutants of *Escherichia coli*. J. Mol. Biol. **60**:65–74.