JOURNAL OF BACTERIOLOGY, July 1986, p. 12–17
0021-9193/86/070012-06$02.00/0

Vol. 167, No. 1

Role and Specificity of Plasmid RP4-Encoded DNA Primase in Bacterial Conjugation

ANDREW MERRYWEATHER,[1] PETER T. BARTH,[2] AND BRIAN M. WILKINS[1]*

Department of Genetics, University of Leicester, Leicester LE1 7RH,[1] and ICI Corporate Bioscience Group, The Heath, Runcorn, Cheshire WA7 4QE,[2] England

Received 22 November 1985/Accepted 28 March 1986

The role of the DNA primase of IncP plasmids was examined with a derivative of RP4 containing Tn*7* in the primase gene (*pri*). The mutant was defective in mediating bacterial conjugation, with the deficiency varying according to the bacterial strains used as donors and recipients. Complementation tests involving recombinant plasmids carrying cloned fragments of RP4 indicated that the primase acts to promote some event in the recipient cell after DNA transfer and that this requirement can be satisfied by plasmid primase made in the donor cell. It is proposed that the enzyme or its products or both are transmitted to the recipient cell during conjugation, and the role of the enzyme in the conjugative processing of RP4 is discussed. Specificity of plasmid primases was assessed with derivatives of RP4 and the $IncI_1$ plasmid ColIb-P9, which is known to encode a DNA primase active in conjugation. When supplied in the donor cell, neither of the primases encoded by these plasmids substituted effectively in the nonhomologous conjugation system. Since ColIb primase provided in the recipient cell acted weakly on transferred RP4 DNA, it is suggested that the specificity of these enzymes reflects their inability to be transmitted via the conjugation apparatus of the nonhomologous plasmid.

Plasmids belonging to the IncP-1 group, such as RP4, are remarkable for their ability to promote conjugation between, and to be maintained within, a broad range of gram-negative bacterial species. One of the best-characterized products specified by RP4 is DNA primase, an enzyme that synthesizes oligoribonucleotides for starting DNA chain growth. The *pri* locus has been mapped, and it is thought to be the penultimate gene in a group of coordinately transcribed genes situated at the end of the Tra1 region of the plasmid (17, 19, 20). The gene encodes two immunologically related polypeptides of 118 and 80 kilodaltons (kDa), and primase activity is specified by a domain located towards the C-terminal region of both products (17, 20). In vitro, the enzyme can generate RNA primers on a variety of single-stranded bacteriophage DNA templates, and it has the potential to substitute in vivo for the primase of *Escherichia coli* (*dnaG* protein) in discontinuous replication of the bacterial chromosome (17, 18, 20).

The physiological role of the primase specified by IncP-1 plasmids is unclear. It was inferred from genetic studies with RP4 *pri* mutants that it optimizes the priming of vegetative replication of the plasmid and that it promotes efficient transconjugant formation in 5 of 11 gram-negative bacterial species tested as recipient strains in conjugation (17). Of these, *Salmonella typhimurium* recipients, but not *E. coli* recipients, were found to be deficient in inheriting Pri^- plasmids. A similar, recipient-specific variability was observed when primase-negative mutants of another IncP-1 plasmid, R18, were tested for conjugation proficiency (16). Thus, the primase apparently contributes to the broad host range of IncP-1 plasmids by facilitating the conjugative metabolism of these DNA elements.

The purpose of our studies was to investigate further the role of RP4-specified primase in bacterial conjugation and to determine whether the enzyme acts in the donor or the recipient cell. A genetic approach was taken, testing for complementation of a primase-negative RP4 mutant by recombinant plasmids carrying a cloned *pri* gene. We also used $IncI_1$ plasmids to assess the specificity of genetically different plasmid primases in bacterial conjugation. The primase encoded by the *sog* gene of $IncI_1$ plasmids such as ColIb-P9 differs from the RP4 enzyme in molecular mass and antigenic specificity, but it is known to act in bacterial conjugation and can generate primers on a similar diversity of phage and bacterial DNA templates as can the RP4 *pri* product (8, 17, 24). In view of these similarities, we examined whether *sog* and *pri* DNA primases are functionally interchangeable in bacterial conjugation.

MATERIALS AND METHODS

Bacterial strains and cultural conditions. Bacterial strains and plasmids are described in Tables 1 and 2. Spontaneous nalidixic acid-resistant mutants of *E. coli* C600 and *S. typhimurium* SL329 were used as recipients. *E. coli* BW103 was isolated as a Thy^+ recombinant of *E. coli* BW85, constructed in a 30-min mating with KL16-99 donors (*thy*$^+$ *recA1* [10]) at 30°C. The recombinant was chosen for its sensitivity to UV light, and the recombination-defective phenotype was confirmed in test matings with HfrH. Plasmid-containing strains were grown in medium containing antibiotics to maintain the presence of the plasmid. Kanamycin (100 μg ml^{-1}) was used for cells harboring pLG261 or derivatives of RP4 and ColIb, tetracycline (7.5 μg ml^{-1}) was used for cells harboring pLG215, chloramphenicol (25 μg ml^{-1}) was used for cells harboring pLG260, and ampicillin (200 μg ml^{-1}) was used for cells harboring pJF107 and its derivative. The permissive temperature for BW86 was 30°C, and thymine-requiring strains were routinely grown in media containing thymine (20 μg ml^{-1}).

Construction of pLG260 and pLG261. The constructions of pLG260 and pLG261 are summarized in Fig. 1. Plasmids pBR328 and R300B were purified by the Triton X-100 method (15), and pRP26 was purified by a procedure involving lysozyme and Brij 58 (23). DNAs were digested with appropriate restriction endonucleases (Bethesda Research Laboratories, Inc.) by the supplier's instructions, treated

* Corresponding author.

with phenol, and precipitated with ethanol. pRP26 fragments and cleaved vector molecules were mixed in a ratio of 1:5 and ligated with T4 DNA ligase (New England BioLabs, Inc.). The resulting DNA was used to transform competent cells of BW86, and transformants were selected at 30°C on nutrient agar containing chloramphenicol for pLG260 and on nutrient agar containing kanamycin for pLG261. After replica plating, replicas were incubated at 40°C to identify recombinant plasmids that suppressed the *dnaG3*(Ts) mutation in BW86. The rapid method of Birnboim and Doly (5) was used for all other plasmid preparations required for mapping and strain construction.

Filter matings. Conjugation experiments involving pRP1- and pRP2-containing donor cells were carried out on solid medium. Cultures of donor and recipient strains, shaken overnight in nutrient broth, were diluted to an A_{600} of 0.35 ($\sim 2.5 \times 10^8$ cells ml^{-1}) and mixed in a 1:5 (vol/vol) ratio. A 1-ml amount of mixture was filtered onto cellulose nitrate disks (Whatman; 25-mm diameter, 0.45-μm pore size), which were placed on prewarmed nutrient agar plates and incubated at 37°C, unless stated otherwise. After 3 h, bacteria were washed from the filters, and the yield of transconjugants was determined by plating on nutrient agar containing nalidixic acid (50 μg ml^{-1}) and either kanamycin or tetracycline. Each result is the average value from at least three experiments.

Conjugative DNA synthesis. Details of conjugative DNA synthesis have been described previously (7). Cells were grown in a salts-glucose-Casamino Acids (Difco Laboratories) (SGC) medium. Recipients were irradiated with UV light (400 Jm^{-2}), and matings were carried out at 41°C in the presence of rifampin (100 μg ml^{-1}), 2-deoxyguanosine (200 μg ml^{-1}), and [2-^{14}C]thymine (63 mCi $mmol^{-1}$, 1 μg ml^{-1}).

Colony formation by BW86. Suitable dilutions of overnight cultures, shaken in nutrient broth at 30°C, were plated on prewarmed nutrient agar plates and incubated at 30°C or 40°C. All media incubated at 30°C contained appropriate antibiotics.

DNA synthesis at 41°C. Overnight cultures were diluted in SGC medium supplemented with thymine (3 μg ml^{-1}) plus 2-deoxyguanosine (200 μg ml^{-1}) and were grown at 30°C for about three mass doublings to an A_{450} of ~0.35. At 5 min after a shift to 41°C, 1.5 ml of culture was added to an equal volume of the same medium containing [*methyl*-^{3}H]thymine to yield 320 mCi $mmol^{-1}$. The A_{450} was determined immediately and after 90 min at 41°C. Radioactivity incorporated into acid-precipitable material in this period is given as counts per minute $\times 10^{-3}$ per unit increase of A_{450}.

TABLE 1. Bacterial strains used

Strain and strain no.	Genotype[a]	Source or reference
E. coli K-12		
W3110T$^-$	*thyA36 deoC*	17
C600	*thr-1 leu-6 thi-1 supE44 lacY1 fhuA21*	17
BW85	*leu thyA deoB rpsL cir*	6
BW86	*leu thyA deoB rpsL cir dnaG3 Δ(chlA-uvrB)*	8
BW96	*leu deoA deoC tdk rpsL cir rpoB*	8
BW103	*leu deoB rpsL cir recA1*	This work
S. typhimurium LT2		
SL329	*trp*	17

[a] Genetic symbols are defined in the linkage map of Bachmann (2).

TABLE 2. Bacterial plasmids used

Plasmid	Description[a]	Source or reference
pLG215	pBR325 Ω(*sog*$^+$ *Eco*RI 8.0 kb) Ap^r Tc^r	24
pLG221	ColIb-P9*drd-1 cib*::Tn5 IncI$_1$ Km^r	8
pLG250	pLG221 carrying *sog-217*	8
pLG260	pBR328 Ω (*pri*$^+$ *Hin*dIII 14.5 kb) Ap^r Cm^r	This work
pLG261	R300B Ω(*pri*$^+$ *Pst*I 12.6 kb) IncQ Km^r	This work
pLG270	ColIb-P9*drd-2 tra*::Tn5 IncI$_1$ Km^r	See text
pRP1	RP4 *pri*::Tn7 IncP-1 Ap^r Tc^r Km^r Tp^r Sm^r Sp^r	3
pRP2	RP4::Tn7 Tra$^+$ IncP-1 Ap^r Tc^r Km^r Tp^r Sm^r Sp^r	3
pRP26	RP4 *tra*::Tn7 IncP-1 Ap^r Tc^r Km^r Tp^r Sm^r Sp^r	3
pJF107	pBR325 Ω(*pri*$^+$ *Hin*dIII 14.5 kb Δ10.6 kb) Ap^r Cm^r	20
pJF107Δ32	pBR325 Ω(*pri*$^+$ *Hin*dIII 14.5 kb Δ~13.7 kb) Ap^r	20

[a] Abbreviations for drug resistance phenotypes are: Ap, ampicillin; Tc, tetracycline; Km, kanamycin; Cm, chloramphenicol; Tp, trimethoprim; Sm, streptomycin; and Sp, spectinomycin.

RESULTS

Experimental plan and bacterial plasmids. We assessed the requirement for primase in conjugation from the yield of *E. coli* K-12 and *S. typhimurium* transconjugants generated in matings mediated by a primase-negative mutant of RP4. The mutant plasmid used was pRP1, which carries a Tn7 insertion preventing the synthesis of an active primase and of the 118-kDa *pri* polypeptide (17). The transposon insertion is about 1.5 kilobases (kb) from the *Hin*dIII site in RP4 (Fig. 1) and therefore is located in the 3′ region of *pri*, as shown on

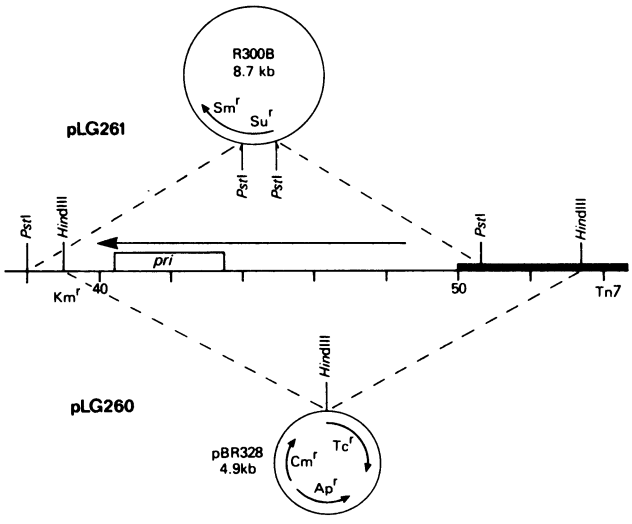

FIG. 1. Construction of *pri*$^+$ recombinant plasmids pLG260 and pLG261. The central linear map shows the *pri* region of pRP26 (RP4::Tn7). The map coordinates of RP4, the location of *pri*, and the putative transcript (line with arrowhead) are taken from the data of Lanka et al. (19, 20). Details of the Tn7 region (thick line) are taken from the data of Gosti-Testu et al. (11). Plasmid pRP1 is an RP4 derivative containing a Tn7 insertion at about coordinate 40.5 (17, 19).

TABLE 3. Plasmid-mediated suppression of the *E. coli dnaG3* mutation

Plasmid in BW86	Colony formation[a]	DNA synthesis[b]
pLG215	1.5×10^{-4}	116
pLG221	2.2×10^{-1}	233
pLG250	$<10^{-7}$	10
pLG260	5.9×10^{-1}	208
pLG261	1.3×10^{-2}	137
pLG270	1.1	261
pRP1	$<10^{-7}$	12
pRP2	2.2×10^{-6}	82
pRP26	3.0×10^{-5}	100
pJF107	3.7×10^{-3}	96
pJF107Δ32	5.1×10^{-5}	85

[a] Colony formation at 40°C relative to that at 30°C. Value of $<10^{-7}$ for BW86.
[b] Value of 10 for BW86. Synthesis was at 41°C.

the 60-kb map of RP4 (19). The control plasmid was pRP2; this RP4 derivative has Tn*7* inserted near the tetracycline resistance (Tc^r) gene in a region that is devoid of known transfer or maintenance genes (3).

Complementation studies involving pRP1 and Pri^+ recombinant plasmids were undertaken to determine whether the primase acts to promote events in the donor or in the recipient cell, and Pri^+ recombinants based on R300B and pBR328 were constructed to this end. R300B is an 8.7-kb plasmid of the IncQ group (13), which is maintained in a broad range of bacterial species, including *S. typhimurium*, and it was used as the vector for the establishment of the cloned *pri* gene in recipient strains. However, R300B is an unsuitable vector for studying the effect of cloned genes in donor cells, because it is mobilized by RP4 to recipient cells during conjugation (4). Thus, the nonmobilizable plasmid pBR328 (22) was used in the construction of a Pri^+ recombinant for the necessary complementation tests in donor cells.

The source of DNA for cloning the *pri* gene was pRP26. This plasmid is an RP4 derivative containing Tn*7* near the putative promoter of the transcriptional unit containing *pri*, and the mutant has merit in that it specifies overproduction of primase (17) and the inserted transposon provides useful restriction sites for the cloning of the adjacent DNA. The constructions of pLG260 and pLG261 are summarized in Fig. 1. The *pri* gene in each recombinant is presumably expressed from the normal RP4 promoter, but the promoter of the sulfonamide resistance-streptomycin resistance (Su^r-Sm^r) operon in the R300B vector (4) may also contribute to the expression of *pri* in pLG261. The ability of these plasmids to suppress the temperature-sensitive *dnaG3* mutation in BW86, as judged by rescue of colony formation and DNA synthesis at high temperature, is shown in Table 3. These parameters provide a good estimate of plasmid primase activity (20, 24). Although pLG260 apparently specifies more primase than does pLG261, both recombinants directed the synthesis of greater amounts of primase than did pRP2, the control plasmid used in the conjugation experiments. Presumably, these differences in primase activity reflect the different copy numbers of the plasmids.

Requirement for plasmid primase in the recipient cell. Initial experiments involved W3110T$^-$ donor cells mated with *E. coli* C600 and *S. typhimurium* SL329 recipients, since these strains were used previously (17). About 5×10^8 pRP2 transconjugants ml^{-1} were obtained from the *E. coli* matings, but the yield was approximately four orders of magnitude lower when *S. typhimurium* was the recipient species. The yields of pRP1 transconjugants relative to pRP2 transconjugants in matings of W3110T$^-$ donors and recipient strains C600, C600(pLG261), SL329, SL329(pLG261), and SL329(R300B) were 0.59, 0.84, 0.01, 0.37, and 0.03, respectively. Thus, formation of pRP1 transconjugants was slightly and reproducibly reduced relative to that of pRP2 transconjugants when C600 was the recipient strain, but a 100-fold reduction was detected in the *S. typhimurium* strain. These deficiencies were mostly overcome when the recipients contained the Pri^+ recombinant pLG261.

These results confirm previous findings (17) that the deficiency of pRP1 transconjugants depends on the strain used as the recipient. Furthermore, the complementation of pRP1 by pLG261 implies that the RP4 mutant fails to specify one or more gene products that promote some event in the recipient cell and that plasmid primase is one such product.

Contribution of plasmid primase in the donor cell. The apparent requirement for plasmid primase in the recipient cell might normally be satisfied by enzyme that is either supplied by the donor or synthesized in the recipient after expression of the transferred DNA. To distinguish between these possibilities, we determined whether the Pri^+ recombinant pLG260 can complement pRP1 in the donor cell. This pBR328-based recombinant was not mobilized by pRP1 or pRP2 (data not shown). W3110T$^-$ proved to be an unsuitable donor strain for these experiments because it was transformed inefficiently by pLG260 and because most transformants were found to contain deletion mutants of the plasmid. BW86 was used instead, since it allowed the presence of a Pri^+ plasmid to be monitored by the rapid method of *dnaG* suppression.

In contrast to results obtained with W3110T$^-$, BW86 donors of pRP1 were almost equally deficient (>94%) in contributing to transconjugants in *E. coli* C600 recipients as they were in *S. typhimurium* SL329 recipients (Table 4). The deficiency in BW86 × C600 matings was not due to mating at 30°C, the permissive temperature for BW86, as indicated by control matings with W3110T$^-$ donors at 30°C and 37°C (data not shown). Furthermore, the deficiency cannot be attributed to the *dnaG3* mutation in BW86 because a dna^+ donor strain (BW85) of the same pedigree gave similar results. The yield of pRP1 transconjugants generated by donors of a third K-12 pedigree (C600) was intermediate between yields obtained with W3110T$^-$ and BW86 strains, confirming that the genotype of the *E. coli* K-12 donor cells influences formation of Pri^- plasmid transconjugants in K-12 recipients. Finally, Table 4 shows that the presence of the Pri^+ recombinant

TABLE 4. Relative yield of pRP1 transconjugants generated by BW86, BW85, and C600 donor cells

Bacterial strains		Temp (°C)	Yield of pRP1 transconjugants relative to pRP2 matings[b]
Donor[a]	Recipient		
BW86	C600	30	0.016
BW86	SL329	30	0.06
BW85	C600	30	0.008
C600	C600	37	0.16
C600	SL329	37	0.032
BW86	C600(pLG261)	30	0.28
BW86	SL329(pLG261)	30	0.58

[a] Each donor strain contained either pRP1 or pRP2 as the conjugative plasmid.
[b] Yield of pRP1 transconjugants expressed as a fraction of the yield of pRP2 transconjugants.

plasmid (pLG261) in the recipient strains enhanced the yield of pRP1 transconjugants generated by BW86 donor cells. This indicates that BW86(pRP1) donor cells resemble W3110T$^-$ donors (see the previous section) in failing to specify some product that acts in the recipient cell.

BW103 containing the nonmobilizable, Pri$^+$ recombinant plasmid pLG260 and either pRP1 or pRP2 was used as the donor strain to test whether the requirement for plasmid primase in pRP1 transconjugant formation can be satisfied by enzyme supplied by the donor. BW103 is homogenic with BW86 and was chosen for its Rec$^-$ phenotype to limit recombination in the donor strain between the cloned RP4 DNA and the homologous region in pRP1. The presence of pLG260 in the donors rescued the deficiency of pRP1 transconjugants in *E. coli* and *S. typhimurium*, as shown in Table 5. This complementation was efficient, since little or no further rescue was detected if the recipients also contained functional primase specified by a Pri$^+$ recombinant plasmid. To ensure that primase, rather than the product of some other RP4 gene product, was responsible, the complementation tests were repeated with Pri$^+$ recombinant plasmids pJF107 and pJF107Δ32, constructed by Lanka et al. (20). pJF107Δ32 contains only the *pri* gene, whereas pJF107 carries this gene and the promoter-distal DNA up to the *Hin*dIII site at coordinate 39 in Fig. 1. These two recombinants complemented pRP1 in the donor with moderate but approximately equal efficiency, implying that the conjugation deficiency of pRP1 is not due to a polar effect of the transposon insertion on the expression of the gene downstream of *pri*. However, neither pJF plasmid complemented pRP1 with the efficiency of pLG260. Since pLG260 apparently specifies more primase activity than do the pJF plasmids (Table 3), presumably because of the fact that the *pri* genes in the two sets of recombinants are expressed from different promoters, the different complementation efficiencies may reflect the amount of primase in the donor cells.

It is interesting that neither pLG260 nor the pJF plasmids complemented pRP1 fully, despite the indication that they specify at least as much plasmid primase as the control RP4 derivative, pRP2 (Table 3) (20). Since the Tn*7* insertion in pRP1 maps at the 3′ end of *pri*, the mutant may encode truncated polypeptides. Competition between such inactive *pri* polypeptides and proteins specified in *trans* may explain the inability of the recombinants to fully complement pRP1.

Complementation of pRP1 by plasmids carrying the *sog* gene of ColIb. Complementation of pRP1 was detected when the recipient strain harbored a Sog$^+$ IncI$_1$ plasmid (pLG221),

TABLE 5. Complementation of pRP1 in the donor strains by nonmobilizable Pri$^+$ recombinant plasmids

Bacterial strains		Yield of pRP1 transconjugants relative to pRP2 matings[b]
Donor[a]	Recipient	
BW103	C600	0.002
BW103(pBR328)	C600	0.004
BW103(pLG260)	C600	0.63
BW103(pLG260)	C600(pLG260)	0.85
BW103(pJF107)	C600	0.15
BW103(pJF107Δ32)	C600	0.19
BW103	SL329	0.067
BW103(pLG260)	SL329	1.6
BW103(pLG260)	SL329(pLG261)	0.71

[a] Each donor also carried either pRP1 or pRP2.
[b] Yield of pRP1 transconjugants as a fraction of the yield of pRP2 transconjugants.

TABLE 6. Tests for complementation of pRP1 by ColIb derivatives

Bacterial strains		Yield of pRP1 transconjugants relative to pRP2 matings[b]
Donor[a]	Recipient	
BW103	C600(pLG221)	0.15
BW103	C600(pLG250)	0.02
BW103	C600(pLG215)	0.008
W3110T$^-$	SL329(pLG221)	0.33
W3110T$^-$	SL329(pLG250)	0.06
BW103(pLG270)	C600	0.005

[a] Each donor also carried either pRP1 or pRP2.
[b] Yield of pRP1 transconjugants as a fraction of the yield of pRP2 transconjugants.

with the extent of the rescue depending on the strains used (Table 6). This complementation is attributed to the activity of *sog* primase, because no such effect was observed when the recipient cells carried a primase-negative mutant of pLG221, namely, pLG250. Interestingly, pRP1 was not complemented by a small recombinant plasmid (pLG215) containing a cloned *sog* gene, suggesting that other ColIb-specified products are required to allow *sog* primase to act on transferred RP4 DNA.

In contrast, no complementation of pRP1 was observed when the donor strain harbored a Sog$^+$ IncI$_1$ plasmid, pLG270. This is a mutant of ColIb*drd-2* that contains a normal *sog* primase gene (Table 3), but it is more than 10^5-fold deficient in conjugation as a result of a Tn*5* insertion (C. Rees and B. M. Wilkins, manuscript in preparation). The plasmid was chosen here for its conjugation deficiency to ensure that *sog* primase was only synthesized in the donor cell. Furthermore, it was used to ensure that, subject to effects of the Tn*5* insertion, other ColIb-specified products that may cooperate with *sog* primase would be present in the donor cell.

Specificity of RP4 primase in conjugation. It was shown previously that *sog* primase of ColIb functions to promote the synthesis of DNA complementary to the transferred IncI$_1$ plasmid strand (8), and we examined whether RP4 primase can substitute for the *sog* product in this process. The method involved mating donors of a ColIb plasmid with rifampin-treated, heavily UV-irradiated BW86 recipients at 41°C. In response to ColIb transfer, such recipients incorporate exogenously supplied, labeled thymine into plasmid DNA by a process dependent on *sog* primase, and this incorporation is readily detectable if uptake of label into DNA of donor cells is prevented by use of a *tdk* strain (7, 8).

Figure 2 shows [^{14}C]thymine incorporation under such conditions when the donors harbored the control Sog$^+$ plasmid pLG221, and it shows the deficiency when its primase-negative derivative pLG250 was used instead. Conjugative synthesis of pLG250 in recipient cells is complemented efficiently in this system by the presence of the pBR325-*sog*$^+$ recombinant, pLG215, in the donor strain (25), but to optimize detection of any effect caused by the pBR328-*pri*$^+$ recombinant (pLG260), the complementation tests were done with the recombinant plasmids present in both the donor and the recipient cells. The results (Fig. 2) indicate that, whereas pLG215 complemented pLG250 fully, pLG260 had no such effect. When pRP26, the RP4 derivative that specifies overproduction of primase, was introduced into the donor cells carrying pLG250, the deficiency of conjugative DNA synthesis in the recipients was only marginally rectified. It should be noted that, since pRP26 is

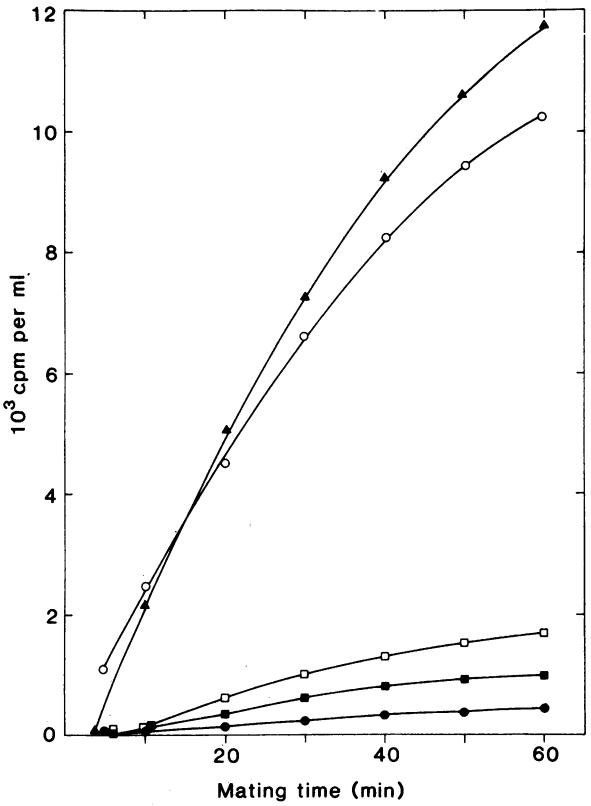

FIG. 2. Primase-dependent conjugative synthesis of ColIb DNA in recipient cells. BW96 and BW86 were used as donor and recipient strains, respectively. Plasmids present were: ○, pLG221 (Sog^+) in donor strains; ●, pLG250 (Sog^-) in donor strains; ▲, pLG250 and pLG215 (Sog^+) in donor strains with pLG215 in recipient strains; ■, pLG250 and pLG260 (Pri^+) in donor strains with pLG260 in recipient strains; □, pLG250 and pRP26 (Pri^+) in donor strains. Values have been corrected by the subtraction of counts per minute incorporated (<400 cpm ml^{-1}) by donor and recipient strains incubated separately.

conjugation defective and was not mobilized by the $IncI_1$ plasmids used here (data not shown), the amount of conjugative DNA synthesis due to the transfer of pRP26 is negligible in such an experiment.

DISCUSSION

The results show that pRP1 was consistently defective in generating transconjugant colonies, but the value varied according to the strains and genera participating in conjugation. A deficiency of ~20-fold or more was detected in *E. coli* K-12 × *S. typhimurium* matings, but, depending on the donor strain used in K-12 × K-12 matings, the deficiency ranged from 1.7 to in excess of 100. Since pRP1 was maintained in both K-12 and *S. typhimurium* with comparable and nearly normal frequencies (17), the primase-negative mutant must be defective in mediating some aspect of conjugation rather than in subsequent vegetative replication required for formation of the transconjugant colony.

We investigated the nature of the conjugative defect of pRP1 through complementation tests. These indicated that the defect is caused by lack of RP4 DNA primase and that any possible polarity effect of the Tn*7* insertion in *pri* on the expression of the gene downstream of the insert is not responsible. Although it was impossible to quantify pRP1 DNA transfer itself in these genetic experiments, it is evident that substantial transfer of the mutant plasmid occurred from donors lacking the plasmid primase and that the enzyme is required to promote establishment of the transferred DNA in the recipient cell. However, the requirement for plasmid primase in the recipient cell was satisfied by enzyme specified in the donor by a nontransferable *pri* gene. This leads to the important conclusion that plasmid primase or its RNA products or both are transmitted from the donor cell to promote some aspect of the conjugative metabolism of RP4 in the recipient cell. Presumably, this reflects the normal mode of action of IncP plasmid primase.

The role of plasmid primase in the recipient cell will remain uncertain until the nature of the transferred RP4 DNA is determined. Like the transfer of IncF and $IncI_1$ plasmids, RP4 transfer is a unidirectional process that is initiated at a specific site on the plasmid (26). It is known that a specific single strand of IncF and $IncI_1$ plasmids is transmitted during conjugation (26), and, if this also applies to RP4 transfer, then an obvious role of the plasmid primase is the generation of primers for complementary strand synthesis in the recipient cell. However, we calculate from the data of Lanka and Barth (17) that RP4 specifies synthesis of about 5,500 molecules of the 118-kDa *pri* polypeptide $cell^{-1}$. This abundance suggests that the protein may perform another function apart from primer generation itself.

Using a different experimental approach, we showed previously that the DNA primase specified by the *sog* gene of ColIb acts analogously in conjugation (8). Furthermore, there is functional and physical evidence that *sog* primase is transferred during conjugation to the recipient cell (9; A. Merryweather, manuscript in preparation). Although RP4 and ColIb primases are different polypeptides, they are similar in their abilities to act in vitro on the single-stranded DNA of phages fd, G4, and φX174, to suppress the *dnaG3* mutation in *E. coli*, and to synthesize oligoribonucleotides of similar composition and size distribution (17, 18, 24). Despite these functional similarities, the two enzymes act specifically during bacterial conjugation since neither primase supplied in the donor cell functioned effectively in the nonhomologous conjugation system, even under conditions in which putative accessory proteins necessary for activity of the nonhomologous primase were likely to be present. However, *sog* primase in the recipient cell acted weakly on transferred RP4 DNA, provided that the enzyme was specified by a ColIb plasmid and not by a cloned fragment. Taken together, these results imply that *sog* primase can function on the transferred strand of RP4 in a process requiring other ColIb-encoded factors but that the various ColIb components cannot be transmitted from the donor cell via the conjugation apparatus of RP4.

There is no absolute requirement for RP4 plasmid primase in bacterial conjugation. In the absence of the enzyme, we assume that primer-generating enzymes native to the recipient cell can substitute, as indicated by the recipient-dependent variation of the relative yield of pRP1 transconjugants. However, the genotype of the donor can influence this substitution, since one K-12 strain ($W3110T^-$) contributed to a relatively normal yield of pRP1 transconjugants in matings with K-12 recipients, whereas strains of the BW86 pedigree lacked this ability. The nature of these complex interactions is unknown, but the important point is that they are normally bypassed by plasmid primase. It may be significant that the chromosome of $W3110T^-$ carries an inversion between *rrnD* and *rrnE* (14) which may lead to altered levels of bacterial primase or accessory proteins.

Why is RP4 primase required for efficient bacterial conjugation yet essentially redundant for vegetative replication of the plasmid? These differences can be reconciled if it is assumed that, like plasmid F (26), a specific strand of RP4 is transferred during conjugation with the 5′ terminus leading. As RP4 replication is unidirectional, only one strand will support formation of multiple primers for discontinuous DNA synthesis. Presumably, this strand contains sites that are recognized by bacterial primer-generating enzymes, possibly acting in conjunction with accessory proteins encoded by regions of RP4 that are nonessential for conjugation. The second strand is likely to be deficient in sites recognized by vegetative priming systems because it supports leading-strand synthesis in DNA replication. Since the direction of RP4 transfer is opposite to that of DNA replication (1, 12, 21), it follows that this second strand is the one, as we have proposed, that is transmitted during conjugation. Hence, plasmid primase provides a conjugation-specific priming mechanism that ensures efficient conversion of the transferred strand into duplex DNA in the recipient cell, thus providing the substrate for vegetative DNA replication.

ACKNOWLEDGMENTS

A.M. was supported by an SERC CASE award in cooperation with ICI Corporate Bioscience Group.

We thank Erich Lanka for supplying pJF107 and pJF107Δ32.

LITERATURE CITED

1. **Al-Doori, Z., M. Watson, and J. Scaife.** 1982. The orientation of transfer of the plasmid RP4. Genet. Res. **39**:99–103.
2. **Bachmann, B. J.** 1983. Linkage map of *Escherichia coli* K-12, edition 7. Microbiol. Rev. **47**:180–230.
3. **Barth, P. T., N. J. Grinter, and D. E. Bradley.** 1978. Conjugal transfer system of plasmid RP4: analysis by transposon *7* insertion. J. Bacteriol. **133**:43–52.
4. **Barth, P. T., L. Tobin, and G. S. Sharpe.** 1981. Development of broad host-range plasmid vectors, p. 439–448. *In* S. B. Levy, R. C. Clowes, and E. L. Koenig (ed.), Molecular biology, pathogenicity, and ecology of bacterial plasmids. Plenum Publishing Corp., New York.
5. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513–1523.
6. **Boulnois, G. J., M. J. Beddoes, and B. M. Wilkins.** 1979. Rifampin disrupts conjugal and chromosomal deoxyribonucleic acid metabolism in *Escherichia coli* K-12 carrying some IncIα plasmids. J. Bacteriol. **138**:324–332.
7. **Boulnois, G. J., and B. M. Wilkins.** 1978. A ColI-specified product, synthesized in newly infected recipients, limits the amount of DNA transferred during conjugation of *Escherichia coli* K-12. J. Bacteriol. **133**:1–9.
8. **Chatfield, L. K., E. Orr, G. J. Boulnois, and B. M. Wilkins.** 1982. DNA primase of plasmid ColIb is involved in conjugal DNA synthesis in donor and recipient bacteria. J. Bacteriol. **152**:1188–1195.
9. **Chatfield, L. K., and B. M. Wilkins.** 1984. Conjugative transfer of $IncI_1$ plasmid DNA primase. Mol. Gen. Genet. **197**:461–466.
10. **Clarke, S. J., B. Low, and W. Konigsberg.** 1973. Isolation and characterization of a regulatory mutant of an aminoacyl-transfer ribonucleic acid synthetase in *Escherichia coli* K-12. J. Bacteriol. **113**:1096–1103.
11. **Gosti-Testu, F., V. Norris, and J. Brevet.** 1983. Restriction map of Tn*7*. Plasmid **10**:96–99.
12. **Grinter, N.** 1981. Analysis of chromosome mobilization using hybrids between plasmid RP4 and a fragment of bacteriophage λ carrying IS1. Plasmid **5**:267–276.
13. **Grinter, N. J., and P. T. Barth.** 1976. Characterization of SmSu plasmids by restriction endonuclease cleavage and compatibility testing. J. Bacteriol. **128**:394–400.
14. **Hill, C. W., and B. W. Harnish.** 1981. Inversions between ribosomal RNA genes of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **78**:7069–7072.
15. **Katz, L., D. T. Kingsbury, and D. R. Helinski.** 1973. Stimulation by cyclic adenosine monophosphate of plasmid deoxyribonucleic acid replication and catabolite repression of the plasmid deoxyribonucleic acid-protein relaxation complex. J. Bacteriol. **114**:577–591.
16. **Krishnapillai, V., J. Nash, and E. Lanka.** 1984. Insertion mutations in the promiscuous IncP-1 plasmid R18 which affect its host range between *Pseudomonas* species. Plasmid **12**:170–180.
17. **Lanka, E., and P. T. Barth.** 1981. Plasmid RP4 specifies a deoxyribonucleic acid primase involved in its conjugal transfer and maintenance. J. Bacteriol. **148**:769–781.
18. **Lanka, E., and J. P. Fürste.** 1984. Function and properties of RP4 DNA primase, p. 265–280. *In* U. Hübscher and S. Spadari (ed.), Proteins involved in DNA replication. Plenum Publishing Corp., New York.
19. **Lanka, E., R. Lurz, and J. P. Fürste.** 1983. Molecular cloning and mapping of *Sph*I restriction fragments of plasmid RP4. Plasmid **10**:303–307.
20. **Lanka, E., R. Lurz, M. Kröger, and J. P. Fürste.** 1984. Plasmid RP4 encodes two forms of a DNA primase. Mol. Gen. Genet. **194**:65–72.
21. **Meyer, R., and D. R. Helinski.** 1977. Unidirectional replication of the P-group plasmid RK2. Biochim. Biophys. Acta **478**: 109–113.
22. **Soberon, X., L. Covarrubias, and F. Bolivar.** 1980. Construction and characterization of new cloning vehicles. IV. Deletion derivatives of pBR322 and pBR325. Gene **9**:287–305.
23. **Uemura, H., and K. Mizobuchi.** 1982. Genetic and physical characterization of the ColIb plasmid using ColIb-R222 hybrids. Mol. Gen. Genet. **185**:1–12.
24. **Wilkins, B. M., G. J. Boulnois, and E. Lanka.** 1981. A plasmid DNA primase active in discontinuous bacterial DNA replication. Nature (London) **290**:217–221.
25. **Wilkins, B. M., L. K. Chatfield, C. C. Wymbs, and A. Merryweather.** 1985. Plasmid DNA primases and their role in bacterial conjugation, p. 585–603. *In* D. R. Helinski, S. N. Cohen, D. B. Clewell, D. A. Jackson, and A. Hollaender (ed.), Plasmids in bacteria. Plenum Publishing Corp., New York.
26. **Willetts, N., and B. Wilkins.** 1984. Processing of plasmid DNA during bacterial conjugation. Microbiol. Rev. **48**:24–41.