JOURNAL OF BACTERIOLOGY, Aug. 1986, p. 703–710
0021-9193/86/080703-08$02.00/0

Vol. 167, No. 2

# Genetic Organization of Plasmid R1162 DNA Involved in Conjugative Mobilization

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Received 13 November 1985/Accepted 2 May 1986

**DNA involved in the mobilization of broad-host-range plasmid R1162 was localized to a region of 2.7 kilobases within coordinates 3.4 to 6.1 kilobases on the R1162 map. By examining the transfer properties of plasmids containing cloned fragments of DNA from within this region, we showed that at least four *trans*-active products and a *cis*-active site (*oriT*) were involved in mobilization. A cloned DNA fragment of 155 base pairs was capable of providing full *oriT* activity. This fragment was located within 600 base pairs of DNA containing the origin of replication of R1162, and its nucleotide sequence and that of neighboring DNA were determined. Activation of *oriT* required R1162-encoded, *trans*-acting products. Deletions which resulted in the loss of one or more of these had a variable effect on transfer efficiency and indicated the presence of both essential and nonessential Mob products. Regions encoding these products flanked *oriT* and in one case appeared to overlap a gene essential for plasmid replication. The implications of these findings with respect to the broad host range of R1162 are discussed.**

R1162 is a small (8.7-kilobase-pair [kbp]), high-copy-number plasmid encoding resistance to sulfonamides and streptomycin (2). Although not self-transmissible, it is efficiently mobilized by certain conjugative plasmids, such as RP4 (RK2), R751, and other members of the P-1 incompatibility group (25; and unpublished observations). R1162 has a broad host range and may be readily transferred to and from many different species of gram-negative bacteria. Therefore, the mechanism of conjugative mobilization is of special interest.

For large, self-transmissible plasmids such as F, R100, and R64, a single strand of plasmid DNA is linearly transferred from donor to recipient cells during conjugation (for a review, see references 35 and 36). It has been generally assumed that the mechanism of transfer of mobilizable plasmids is similar. Like the self-transmissible plasmids, the mobilizable plasmids ColE1 (34), CloDF13 (32), pSC101, (A. Nordheim, Ph.D. thesis, Freie Universitat, Berlin, 1979), and RSF1010 (36) (probably identical to R1162 [12]) have a small segment of DNA required in *cis* for mobilization. The site of DNA nicking by the plasmid relaxation complex (3, 28) is also within this DNA (34) and is believed to be at the origin of the transferred strand. This is consistent with the observation that mutations affecting the relaxation complex also generally affect mobilization, and vice versa (9). The relaxation complex has also been identified for some self-transmissible plasmids (14, 27, 28). In the case of RK2, the nicking site is very close to or at the origin of transfer (14). Site-specific nicking of DNA containing the F origin of transfer has also been demonstrated (10).

In addition to the origin of transfer, mobilization requires plasmid-encoded proteins, although little is known about their properties. Some of these may be components of the relaxation complex (9, 17, 22). ColE1 probably encodes a 16,000-dalton species; in addition, a 60,000-dalton polypeptide, which may or may not be plasmid encoded, becomes covalently linked to the 5′ end of the nicked strand (13).

We cloned and mapped those regions of R1162 DNA that are required for efficient mobilization by the IncP1 plasmid R751. A region required in *cis*, *oriT*, is flanked by coding sequences for at least four products active in *trans* for mobilization. Not all of these products are essential for mobilization of R1162, but all affect, to a greater or lesser degree, the efficiency of this process in *Escherichia coli*. In this paper we present the evidence for these conclusions and discuss their significance with regard to the broad host range of R1162.

## MATERIALS AND METHODS

**Bacterial strains and medium.** The *E. coli* K-12 strains used in this study were MV10 (Δ*trpE5*) (15), MV12 (Δ*trpE5 recA56*) (15), and DF1019 (nalidixic acid-resistant [Nal$^r$]) (11), all of which are derivatives of strain C600 (*thr leu lacY thi supE44 fhuA*), and RB791 (W3110 *lacI*$^q$ L8) (1). Cells were grown in TYE (1% tryptone, 0.5% yeast extract, 0.5% NaCl) supplemented as required with carbenicillin, trimethoprim (each 200 μg/ml), chloramphenicol, nalidixic acid, streptomycin sulfate, and tetracycline hydrochloride (each at a concentration of 25 μg/ml). For the selection of carbenicillin-resistant transconjugants, the medium also contained methicillin (4 mg/ml) to inhibit the background growth of sensitive cells (37).

**Plasmids and cloning procedures.** The plasmids used in this study and their derivations are described in Table 1. Most of these plasmids consist of fragments of R1162 DNA cloned into the vectors pBR322 (4) or pACYC184 (7). Throughout the text, the R1162 DNA present in these derivatives, expressed in kilobase-pair coordinates on the standard R1162 map (Fig. 1), is generally shown in parentheses after the plasmid designation.

The objective of most of our cloning efforts was to obtain families of R1162 DNA fragments, each family consisting of members with deletions extending a variable distance from a fixed point. The sets of desired deletions were obtained by partial digestion of plasmid DNAs with BAL 31 exonuclease or with the restriction enzyme *Hpa*II (Table 1).

**Bacterial matings and measurement of plasmid transfer frequencies.** In experiments involving conjugative mobilization of plasmid R1162, the donor strains were MV10 or MV12 containing one or more test plasmids in addition to

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TABLE 1. Plasmids

| Plasmid | Relevant properties[a] | Reference or construction |
|---|---|---|
| Naturally occurring plasmids and cloning vectors | | |
| R1162 | IncQ(P-4) $Su^r$ $Sm^r$ $Mob^+$ | 2 |
| R751 | IncP1 $Tp^r$ $Tra^+$ | 18 |
| pBR322 | Rep(pMB1) $Cb^r$ $Tc^r$ | 4 |
| pACYC184 | Rep(p15) $Cm^r$ $Tc^r$ | 7 |
| pUC18 | Rep(pMB1) $Cb^r$ *lacPOZ*(α) | 29 |
| Plasmids containing cloned fragments of R1162 | | |
| pMS64 | $Cb^r$ $Mob^+$ | 25 |
| pMS92, pMS93, pMS94 | $Cb^r$ $RepI^+$ $RepII^+$ | 25 |
| pMS170 | $Cb^r$ | 21 |
| pMS251 | $Cm^r$ | R1162 DNA of pMS170 (5.2 to 6.5) cloned into pACYC184 by replacement of small *Hin*dIII-*Sal*I fragment of vector |
| pMS276 | $Cb^r$ | Replacement of small *Cla*I-*Bam*HI fragment of pBR322 with *Hpa*II-*Mbo*I R1162 fragment (5.82 to 6.5) |
| pMS316 | $Km^r$ *oriV*(R1162) | Satellite plasmid (5.91 to 6.5) constructed as previously outlined (21) |
| pUT302 | $Tc^r$ $RepI^+$ | Insertion of fragment containing R1162 DNA (0.0 to 3.0) into *Eco*RI site of pACYC184 |
| pUT193, pUT194 | $Cb^r$ | Partial digestion of *Cla*I-cleaved pMS64 (3.8 to 6.6) DNA with *Hpa*II to generate family of molecules with deletions extending a variable distance into R1162 DNA from 6.6 end |
| pUT205, pUT206, pUT208 | $Cb^r$ | Same as for pUT193 and pUT194 except that the starting plasmid contained R1162 DNA (3.8 to 6.6) cloned in the opposite orientation; this procedure generated family of molecules with deletions extending a variable distance into R1162 DNA from the 3.8 end |
| pUT213, pUT217, pUT218, pUT221, pUT222 | $Cm^r$ | Partial digestion of DNA fragment, derived from pUT194 and containing R1162 DNA (3.8 to 5.8), with BAL 31 exonuclease, and cloning of remaining DNA by replacement of small *Sal*I-*Eco*RV fragment of pACYC184; this procedure generated family of molecules with deletions extending a variable distance into R1162 DNA from the 5.8 end |
| pUT256 | $Cb^r$ | *Taq*I-digested R1162 DNA cloned into the *Cla*I cleavage site of pBR322 DNA |
| pUT257 | $Cb^r$ | *Hae*III-digested R1162 DNA cloned into the *Eco*RV cleavage site of pBR322 DNA |
| pUT291, pUT293, pUT297, pUT392 | $Cm^r$ | Partial digestion of DNA fragment, derived from pUT217 and containing R1162 DNA (3.8 to 5.48), with BAL 31 exonuclease and cloning of remaining DNA by replacement of small *Sal*I-*Eco*RV fragment of pACYC184; this procedure generated a family of molecules with deletions extending a variable distance into R1162 DNA from the 3.8 end |
| pUT308, pUT425 | $Cb^r$ | Partial digestion of *Cla*I-cleaved pMS94 (0.7 to 5.7) DNA with *Hpa*II to generate a family of molecules with deletions extending a variable distance into R1162 DNA from the 0.7 end |
| pUT333 | $Cb^r$ | Partial digestion of *Cla*I-cleaved pMS170 (5.2 to 6.5) DNA with *Hpa*II to generate a molecule with a deletion extending into R1162 DNA from 5.2 to 5.54 kbp |
| pUT396 | $Cb^r$ | Replacement of the small *Eco*RI-*Bam*HI fragment of pBR322 with R1162 DNA (0.0 to 5.7) containing an *Hae*II-generated internal deletion from 1.1 to 3.4 kbp |
| pUT459 | $Cb^r$ | Replacement of small *Hin*dIII-*Sal*I fragment of pUC18 DNA with *Hin*dIII-*Sal*I fragment, derived from pMS92, containing R1162 DNA (0.7 to 4.4) |

[a] Abbreviations: $Cb^r$, $Cm^r$, $Km^r$, $Sm^r$, $Su^r$, $Tc^r$, and $Tp^r$, resistance to carbenicillin, chloramphenicol, kanamycin, streptomycin, sulfonamides, tetracycline, and trimethoprim, respectively; Rep( ), replicon derived from parental plasmid in parentheses; $RepI^+$ and $RepII^+$, *trans*-acting products required for R1162 replication; *oriV*(*R1162*), contains origin of DNA replication of R1162; $Tra^+$, conjugatively self-transmissible; $Mob^+$, conjugatively mobilizable; Inc, member of indicated incompatibility group.

R751 as the mobilizing vector; exceptions are noted in the text. The recipient strain was DF1019, and nalidixic acid was used to select against the donor cells. Donor and recipient cells were grown to mid-log phase in TYE containing antibiotics to select for all plasmids present, washed, and suspended in 40 μl of TYE at an approximate donor-to-recipient ratio of 1:10 and a final concentration of $2 \times 10^7$ to $7 \times 10^7$ cells per ml. This suspension was spotted onto TYE–1.4% agar medium and incubated at 37°C. After 90 min, we recovered the mating cells by cutting out an agar plug supporting them. These cells were suspended in 1 ml of TYE by vigorous vortexing and were promptly plated at various dilutions onto selective media. Plasmid mobilization frequencies were expressed as the number of transconjugant cells per donor cell. The number of donor cells was taken as the number of trimethoprim-resistant (R751-containing) cells, determined by plating the mating mixture. The transfer frequency of R751 was consistently between 0.1 and 0.01 in

our experiments. Transfer frequencies of plasmids which were mobilized inefficiently ($<10^{-6}$ transconjugants per donor) were calculated from only a few transconjugant colonies, and these frequencies were necessarily approximate.

**Screen for an active RepII region in derivatives of R1162.** For R1162 plasmids to be maintained in the cell, a replicative origin (*oriV*) is required in *cis*, and the products of two regions of the plasmid, RepI (0.9 to 2.6) and RepII (3.4 to 4.4), are required in *trans* (26). To determine whether a plasmid was RepII$^+$, we asked whether it could support replication of a RepI$^-$ RepII$^-$ *oriV*$^+$ satellite plasmid in a host cell in which RepI products were being provided. We first constructed a strain containing both the test plasmid and pUT302, a pACYC184 derivative which is RepI$^+$ (Table 1), and then transformed this strain with plasmid DNA from cells containing R1162 and the satellite plasmid pMS316, which encodes resistance to kanamycin (Km$^r$). After plating, the emergence of colonies of Km$^r$ cells that did not simultaneously become resistant to streptomycin because of cotransformation with R1162 indicated that the test plasmid was RepII$^+$. In positive cases, this was confirmed by physical examination of the plasmid DNA in the transformants and also by a retransformation procedure: plasmid DNA from one of these transformants was used to transform MV10 (R1162) cells to Km$^r$. If pMS316 had in fact been maintained as a satellite plasmid and the Km$^r$ gene had not been simply rescued by recombination with the resident plasmids, then the Km$^r$ transformants of MV10 (R1162) would in general not acquire the drug resistances of the supporting plasmids.

**Other procedures.** Plasmid DNA was isolated by the rapid boiling method of Holmes and Quigley (16) or by the glass fiber binding method of Marko et al. (23). DNAs were digested with restriction endonucleases and T4 polynucleotide ligase that were purchased from a commercial source. DNA was partially digested with BAL 31 exonuclease by incubating approximately 6 μg of DNA and 1 U of enzyme at 37°C for a total time of 15 min. Samples were taken at intervals of 2.5 min during the course of the digestion. DNA fragments were analyzed by electrophoresis through 0.8 to 1.4% agarose gels or polyacrylamide gels (polymerized from a 5% acrylamide–0.24% bisacrylamide solution). Gels were stained with ethidium bromide and photographed (25). Bacteria were transformed with plasmid DNA by the method of Cohen et al. (8). DNA sequencing was by the dideoxy procedure (30); the DNA fragments to be sequenced were first cloned into the DNA of the M13 derivatives mp8 and mp9 (24).

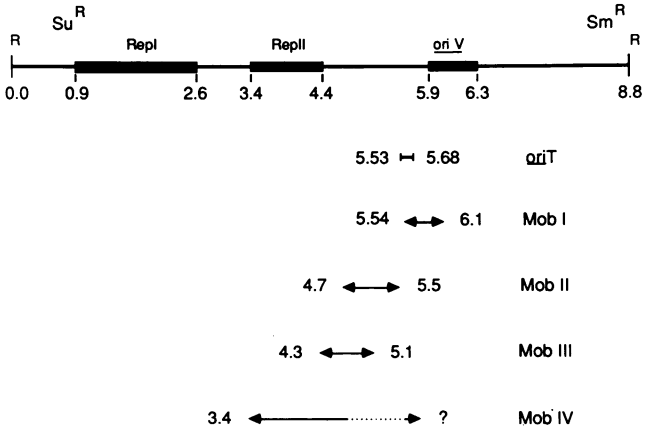


FIG. 1. Standard map of R1162 (above). Locations of genes encoding resistance to sulfonamides (Su$^r$) and streptomycin (Sm$^r$) are indicated. RepI and RepII, regions encoding products required in *trans* for plasmid DNA replication, and the origin of replication (*oriV*) are also shown. The thick lines indicate the maximum size of these regions. Map positions are given in kilobase-pair coordinates from the *Eco*RI cleavage site (R). Locations and maximum sizes for the origin of transfer (*oriT*) and for regions encoding products involved in mobilization (MobI-IV), determined from results described in the text, are shown below.

## RESULTS

**DNA requirement in *cis* for plasmid mobilization maps between 5.53 and 5.68 kbp on the R1162 map.** To identify the R1162 DNA required in *cis* for mobilization, we first generated small fragments of plasmid DNA with restriction endonucleases and cloned these into the vector pBR322. Hybrid molecules were then screened for mobilization from an MV10 donor strain containing both the mobilizing plasmid R751 and R1162, which would provide any plasmid-encoded products necessary in *trans*. Two mobilizable derivatives that we obtained by this procedure were pUT256, which contained a *Taq*I-generated fragment of about 150 base pairs, and pUT257, with an *Hae*III-generated fragment of the same size (Table 2, lines 1 and 3). Both plasmids required R1162 for mobilization (Table 2, lines 2 and 4). A *Taq*I fragment identical in size to that reported here and necessary in *cis* for mobilization of RSF1010 was previously reported (36). We designated the DNA required in *cis* for mobilization *oriT*, although we had not assigned a specific function to it.

We mapped the locations of the *oriT*-containing DNA fragments. From previous results (26), we knew that *oriT* must be located near the replicative origin, *oriV*. We therefore determined the base sequence in the vicinity of *oriV*, and compared this with the sequences of the cloned *oriT* fragments. We found that the *Hae*III fragment was located between 5.52 and 5.68 kbp; the *Taq*I fragment was located between 5.53 and 5.69 kbp (Fig. 2). The replicative origin maps within 5.9 and 6.3 kbp, with essential bases located between 5.9 and 6.1 kbp (26). Thus, *oriT* must lie within 600 base pairs of *oriV*.

**DNA affecting the frequency of mobilization and located on both sides of *oriT*.** In addition to containing *oriT*, pMS64 (3.8 to 6.6 kbp) must encode all R1162-specified products required for mobilization by R751 because it is transferred in the absence of R1162 as helper plasmid (Table 2; reference 25). To begin mapping the additional required DNA, we tested for mobilization plasmids which contained only a part of the 3.8 to 6.6-kbp segment. We found that pUT193 (3.8 to 6.1 kbp) was mobilizable, whereas pUT194 (3.8 to 5.8 kbp) was poorly so (Table 2, lines 6 and 7). In addition, pUT205 (4.0 to 6.6 kbp) was fully mobilizable, pUT206 (4.5 to 6.6 kbp) was mobilized at a low frequency, and pUT208 (4.8 to 6.6 kbp) was not transferred at a detectable level in our assay (Table 2, lines 8 through 10). We concluded that DNA on both sides of *oriT* was necessary for optimal mobilization. This DNA was located within the R1162 coordinates 4.0 to 6.1 kbp. We refer to such DNA, encoding functions able to promote mobilization of *oriT*-containing plasmids, as Mob DNA.

To examine the relationship between the Mob sequences and *oriT*, we carried out mating experiments in which pairs of plasmids, containing different portions of Mob DNA, were tested for mobilization in the presence of R751. The

TABLE 2. Mobilization frequencies of R1162 derivatives

| Test plasmids[a] | Mobilization frequency[b] |
|---|---|
| 1. pUT256 (5.53–5.69), R1162 | $1 \times 10^{-4}$ (pUT256) |
| 2. pUT256 (5.53–5.69) | $<2 \times 10^{-8}$ |
| 3. pUT257 (5.52–5.68), R1162 | $1 \times 10^{-2}$ (pUT257) |
| 4. pUT257 (5.52–5.68) | $<6 \times 10^{-9}$ |
| 5. pMS64 (3.8–6.6) | $6 \times 10^{-3}$ |
| 6. pUT193 (3.8–6.1) | $4 \times 10^{-3}$ |
| 7. pUT194 (3.8–5.8) | $9 \times 10^{-7}$ |
| 8. pUT205 (4.0–6.6) | $2 \times 10^{-3}$ |
| 9. pUT206 (4.5–6.6) | $1 \times 10^{-6}$ |
| 10. pUT208 (4.8–6.6) | $<1 \times 10^{-7}$ |
| 11. pBR322, pMS251 (5.2–6.5) | $<1 \times 10^{-7}$ (pMS251) |
| 12. pUT194 (3.8–5.8), pMS251 (5.2–6.5) | $3 \times 10^{-4}$ (pMS251) |
| 13. pUT194 (3.8–5.8), pMS251 (5.2–6.5) | $3 \times 10^{-3}$ (pUT194) |
| 14. pUT194[c] (3.8–5.8), pMS251 (5.2–6.5) | $4 \times 10^{-4}$ (pMS251) |
| 15. pUT194[c] (3.8–5.8), pMS251 (5.2–6.5) | $1 \times 10^{-3}$ (pUT194) |
| 16. pUT217 (3.8–5.48), pMS170 (5.2–6.5) | $1 \times 10^{-3}$ (pMS170) |
| 17. pUT218 (3.8–5.38), pMS170 (5.2–6.5) | $<4 \times 10^{-8}$ (pMS170) |
| 18. pUT213 (3.8–5.7) | $2 \times 10^{-7}$ |
| 19. pUT333 (5.54–6.5), pUT213 (3.8–5.7) | $5 \times 10^{-5}$ (pUT213) |
| 20. pMS276 (5.82–6.5), pUT213 (3.8–5.7) | $3 \times 10^{-7}$ (pUT213) |
| 21. pUT291 (4.3–5.48), pMS170 (5.2–6.5) | $1 \times 10^{-3}$ (pMS170) |
| 22. pUT293 (4.4–5.48), pMS170 (5.2–6.5) | $2 \times 10^{-5}$ (pMS170) |
| 23. pUT297 (4.7–5.48), pMS170 (5.2–6.5) | $3 \times 10^{-5}$ (pMS170) |
| 24. pUT392 (5.1–5.48), pMS170 (5.2–6.5) | $<2 \times 10^{-7}$ (pMS170) |
| 25. pUT221[c] (3.8–5.1), pUT206 (4.5–6.6) | $9 \times 10^{-4}$ (pUT206) |
| 26. pUT222[c] (3.8–4.5), pUT206 (4.5–6.6) | $1 \times 10^{-6}$ (pUT206) |
| 27. pMS94 (0.7–5.7) | $2 \times 10^{-5}$ |
| 28. pUT425 (3.0–5.7) | $1 \times 10^{-4}$ |
| 29. pUT308 (3.5–5.7) | $4 \times 10^{-7}$ |
| 30. pUT396 (0.0–1.1, 3.4–5.7) | $4 \times 10^{-5}$ |
| 31. pBR322,[d] pUT213 (3.8–5.7) | $<6 \times 10^{-8}$ (pUT213) |
| 32. pMS94[d] (0.7–5.7), pUT213 (3.8–5.7) | $1 \times 10^{-6}$ (pUT213) |
| 33. pUT308[d] (3.5–5.7), pUT213 (3.8–5.7) | $<2 \times 10^{-8}$ (pUT213) |
| 34. pMS92[d] (0.7–4.4), pUT213 (3.8–5.7) | $2 \times 10^{-8}$ (pUT213) |
| 35. pUT459[d] (0.7–4.4), pUT213 (3.8–5.7) | $<2 \times 10^{-8}$ (pUT213) |

[a] Donor strain MV10 also containing R751, except where indicated. The numbers in parentheses after each plasmid indicate the kilobase-pair coordinates of the cloned R1162 DNA.
[b] Number of transconjugants per donor cell for the plasmid in parentheses.
[c] Donor strain MV12 (R751).
[d] Donor strain RB791 (R751).

plasmid pUT194 (3.8 to 5.8) contained *oriT* and part of the Mob DNA cloned into pBR322. Lacking the right end of the Mob sequences, it was mobilized poorly (compare with pUT193 [Table 2, lines 6 and 7]). The plasmid pMS251 (Table 1) was a derivative of pACYC184 containing an R1162 DNA insert with Mob sequences between 5.2 to 6.5 kbp. This plasmid was not detectably mobilized by R751 (Table 2, line 11). From the results presented in this section, it is evident that this was because of missing Mob sequences, to the left of *oriT* and lying between 4.0 and 5.2 kbp. We asked whether both plasmids could be mobilized from the same donor cell; the result (Table 2, lines 12 and 13) showed that this was the case. Mobilization was not due to the reformation of an intact Mob region following ordinary homologous recombination between pMS251 and pUT194, because the same result was obtained when the *recA* donor strain MV12 was used (Table 2, lines 14 and 15). This result did not rule out the possibility that site-specific, *recA*-independent recombination at *oriT*, perhaps one of the functions of the Mob proteins (6), was required to restore mobilization.

What DNA is required for the reconstitution of mobilization? To address this question, we constructed plasmids containing variable amounts of R1162 DNA from pUT194 (3.8 to 5.8 kbp) (Table 1). Each of these new plasmids contained R1162 DNA from 3.8 kbp to a different endpoint, cloned into pACYC184. We then determined which of these plasmids still allowed mobilization of pMS251 when present in the same cell. However, we could not use pMS251 directly, because it was a derivative of pACYC184 and therefore was incompatible with these plasmids. Instead we used pMS170, which contained the identical R1162 DNA present in pMS251, but cloned into pBR322. We observed that pUT217 (3.8 to 5.48 kbp) promoted mobilization of pMS170 (5.2 to 6.5 kbp), whereas the slightly smaller pUT218 (3.8 to 5.38 kbp) was ineffective (Table 2, lines 16 and 17). Therefore, Mob sequences to the left of *oriT* were determined to have an endpoint between 5.38 and 5.48 kbp.

We also carried out a reciprocal experiment to further localize Mob DNA to the right of *oriT*. The plasmid pUT213, like pUT194, lacked part of this Mob DNA and was poorly mobilized (Table 2, line 18). The frequency of mobilization of pUT213 was increased by pUT333 (5.54 to 6.5 kbp) but not pMS276 (5.82 to 6.5 kbp) (Table 2, lines 19 and 20). The left endpoint of this Mob DNA should then have lain between 5.54 and 5.82 kbp. However, because pUT333 may contain some *oriT* DNA, the increased frequency could have been the consequence of site-specific recombination at *oriT* (6). In this case, we could have incorrectly localized the Mob DNA and could also have falsely concluded that the function encoded by this DNA was active in *trans*. We do not believe

that site-specific recombination was involved, both because we were unable to detect by physical means recombinant molecules in the mating population and because pUT333 was never recovered from recipient cells.

**Both essential and nonessential sequences in Mob DNA.** A comparison of the mobilization frequencies of pUT205, pUT206, and pUT208 (Table 2, lines 8 through 10) suggested that some of the DNA between 4.0 and 4.8 kbp, although affecting the efficiency of mobilization, was not required. The data (Table 2, lines 21 through 24) led to a similar conclusion. We constructed plasmids in which a portion of the R1162 DNA present in pUT217 (3.8 to 5.48 kbp) was deleted. Each deletion extended from 3.8 kbp to a variable endpoint. The resulting plasmids were then tested to see whether the remaining R1162 DNA could still promote mobilization of pMS170. A deletion extending from 3.8 to 4.3 kbp did not affect the mobilization frequency of pMS170, whereas larger deletions, ending at 4.4 or 4.7 kbp, resulted in approximately a 100-fold decrease in the transfer frequency of pMS170 (compare pUT291, pUT293, and pUT297 [Table 2, lines 21 through 23]). A still larger deletion, to 5.1 kbp, eliminated DNA essential for mobilization (Table 2, line 24).

The nonessential Mob DNA encoded one or more products active in *trans*. The plasmid pUT206 (4.5 to 6.6 kbp) lacked part of the nonessential Mob sequences and was mobilized poorly (Table 2, line 9). However, the presence of the missing DNA in *trans*, cloned as part of the R1162 DNA in plasmid pUT221 (3.8 to 5.1 kbp), resulted in an increased frequency of mobilization (Table 2, line 25). A similar plasmid, pUT222 (3.8 to 4.5 kbp), which lacked part of the nonessential sequences, failed to boost transfer (Table 2, line 26). We concluded that Mob DNA to the left of *oriT* could be divided into two regions: one mapping within 4.3 and 5.1 kbp and nonessential for mobilization and the second mapping within 4.7 and 5.48 kbp and containing DNA that was essential for mobilization.

**Region of R1162 DNA containing a gene necessary for replication and also involved in mobilization.** Plasmid DNA sequences between 4.3 and 6.1 kbp were necessary for the efficient mobilization of R1162. Plasmids lacking DNA at the 6.1-kbp end were transferred at lower frequency, even when they retained an intact *oriT*. For example, the frequency of mobilization of pUT213 (3.8 to 5.7 kbp) is only $2 \times 10^{-7}$ transconjugants per donor cell (Table 2, line 18). However, pMS94, a derivative of pBR322 containing R1162 DNA between 0.7 and 5.7 kbp, was consistently mobilized at an approximately 100-fold higher frequency than pUT213 (Table 2, line 27). One explanation is that DNA to the left of the Mob region on the R1162 map provided a function that partially compensated for the missing Mob sequences. To test this possibility, we generated a set of deletion derivatives of pMS94 and examined these for transfer. In each derivative, a portion of the R1162 DNA present in pMS94 was deleted, from 0.7 kbp to a variable second endpoint. As expected, deletions extending into the Mob region resulted in a low freqency of mobilization (data not shown). In addition, however, plasmids with deletion endpoints outside the Mob DNA, in the region between 3.5 and 4.3 kbp, were also poorly transferred. For example, although pUT425 (3.0 to 5.7 kbp) was mobilized at a rate comparable to that of pMS94, pUT308 (3.5 to 5.7 kbp) was mobilized at a substantially lower frequency (compare lines 28 and 29, Table 2). We also tested plasmid pUT396 (0.0 to 1.1, 3.4 to 5.7 kbp), which we had constructed by another means (Table 1). The mobilization frequency of this plasmid was similar to that of pMS94 (Table 2, line 30).

The additional DNA contributing to the mobilization of pMS94 overlapped a region of R1162, designated RepII, which contains at least one essential plasmid gene (26). This region maps within 3.4 and 4.4 kbp and is one of two regions (the other, RepI, maps between 0.9 and 2.6 kbp [26]; Fig. 1) which specify products necessary in *trans* for plasmid replication. We tested the derivatives pUT425, pUT308, and pUT396 to see whether they contained an intact RepII region (see Materials and Methods). We found that there was a concomitant loss of both transfer and RepII activities when DNA extending to the right of 3.4 kbp was deleted.

What is the relationship between RepII and the additional function for the mobilization of pMS94? To answer this question, we first showed that the mobilization function was active in *trans*: the plasmid pUT213 transferred very poorly from the donor strain RB791; in the presence of pMS94, the frequency increased at least 100 times (Table 2, lines 31 and 32). A similar increase was not observed in the presence of the plasmid pUT308 (3.5 to 5.7 kbp), similar in structure to pMS94 but lacking R1162 DNA from the RepII region (Table 2, line 33). We next tested whether pMS92 (0.7 to 4.4 kbp) also increased the transfer frequency of pUT213. In this case no increase was observed (Table 2, line 34), although pMS92 was RepII$^+$ (Fig. 3, lanes a and b). This result suggested that the RepII gene product was not responsible for the enhanced mobilization of pMS94. However, we noticed that the copy number of satellite plasmid pMS316 maintained in cells containing pMS92 was much less than that in cells containing pMS94 (Fig. 3; compare lanes a and b with e and f). The difference in satellite plasmid copy number could have been caused by reduced expression of RepII in pMS92, and this could then have accounted for the lack of increased transfer of pUT213. We fused the RepII DNA in pMS92 to the strong *lac* promoter by cloning the R1162 DNA into pUC18 to yield the plasmid pUT459. This plasmid maintained the satellite pMS316 at levels similar to those in strains containing pMS94, and the increased copy number was partially isopropyl-β-D-thiogalactopyranoside-dependent (compare lanes c and d, Fig. 3). Nevertheless, pUT459 still failed to increase the frequency of mobilization of pUT213 (Table 2, line 35). We concluded that the RepII and mobilization functions were not identical. DNA necessary for enhanced mobilization of pMS94 must be located to the right past 5.1 kbp, because pMS93 (0.7 to 5.1 kbp, RepII$^+$), which has the same structure as pMS93 but contains additional R1162 DNA between 4.4 and 5.1 kbp, also failed to increase the frequency of mobilization of pUT213 (data not shown).

## DISCUSSION

The locations of those regions in R1162 which specified products affecting the efficiency of mobilization are summarized at the bottom of Fig. 1. The maximum size of each of these regions, which we designated MobI through MobIV, is shown. We do not have a good estimate for the maximum size of MobIV; however, DNA to the right of 5.1 kbp is required for expression, because pMS93 (0.7 to 5.1 kbp) lacked MobIV activity. Thus, it is likely that the MobIV coding sequence overlaps other genes involved in replication and mobilization. Overlapping coding sequences are associated with the primase genes of RP4 and IncIα plasmids (5, 20). In these cases, there are two overlapping, in-phase reading frames which differ at the amino-terminal coding end. The biological significance of this arrangement is unknown. We conclude from the properties of the RepII-*lac* promoter fusion (Fig. 3) that transcription of the RepII gene

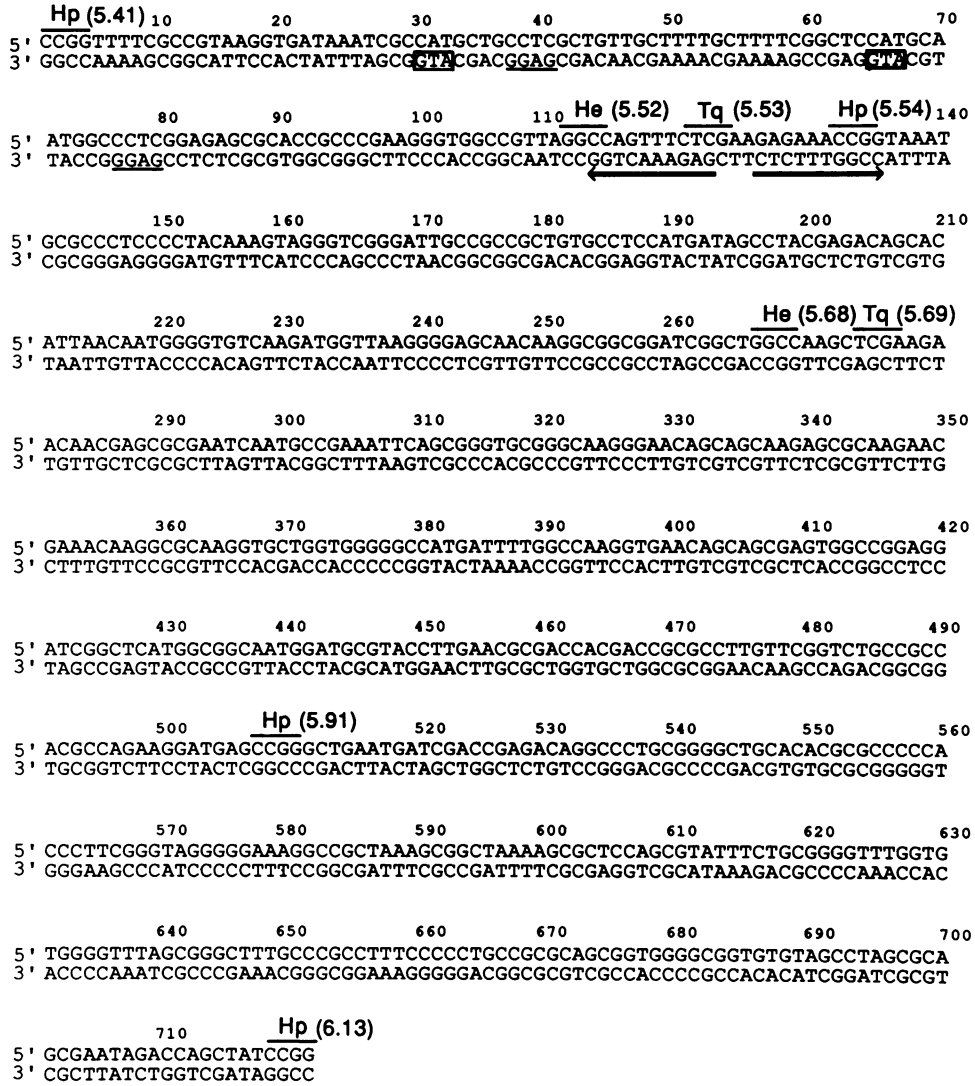


FIG. 2. Nucleotide sequence of R1162 DNA between coordinates 5.41 and 6.13 kbp, including the *Hae*III (5.52 to 5.68) and *Taq*I (5.53 to 5.69) fragments which contain *oriT*. The entire MobI region (Fig. 1 and text) is also located in this DNA, as well as the right end of the Mob DNA mapping to the left of *oriT*. Two possible initiation codons (boxed), each associated with a potential ribosome binding site (underlined), are indicated. Arrows, twofold symmetry in the *oriT* region. Part of the origin of vegetative replication (*oriV*) is located on the *Hpa*II fragment mapping between 5.91 and 6.13 kbp (26). Abbreviations: He, *Hae*III; Hp, *Hpa*II; Tq, *Taq*I. Only relevant restriction enzyme cleavage sites are shown.

is from right to left; perhaps a larger form of the RepII product, requiring sequences further to the right, is involved in mobilization. That gene products for replication and mobilization might be related is not unreasonable, particularly if conjugative mobilization involves DNA synthesis, as it does for self-transmissible plasmids.

A summary of our results (Table 3) illustrates that not all the Mob products are required for mobilization of R1162 by R751 and that the overall frequency of transfer achieved by a particular plasmid depends in a complex way on which Mob functions are present. What accounts for this complexity? One possibility is that some of the Mob genes encode products whose functions partially overlap, either with those of the other Mob genes or with those specified by the mobilizing vector or host. Each gene product would play a role in mating that would depend on the bacterial species and the mobilizing vector, and the resulting adaptability might then contribute to the broad host range of the plasmid. A conditional requirement for a gene product involved in transfer has been demonstrated for both self-transmissible and mobilizable plasmids. Variants of the broad-host-range plasmid RP4 that no longer encode an active primase are defective for transfer into some species but not into others (19). ColE1 also encodes some Mob functions which are required for its mobilization by only some self-transmissible plasmids (33).

The *oriT* region lies within 600 base pairs of the origin of replication (*oriV*). This result agrees with the observations of Nordheim et al. (28), who reported that *oriT* and *oriV* of RSF1010 were within 850 base pairs. Despite their proximity, the two origins of R1162 are functionally independent (our results and reference 26). Sequences sufficient for *oriT* activity were contained within 155-base-pair *Taq*I (5.53 to 5.69 kbp) and *Hae*III (5.52 to 5.68 kbp) fragments. A *Taq*I

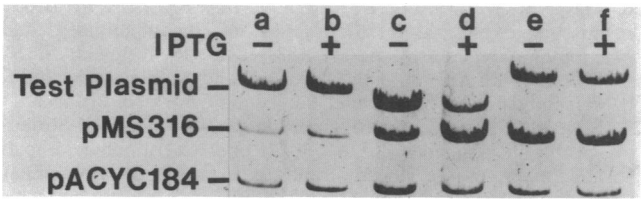


FIG. 3. Agarose gel electrophoresis of *Eco*RI-cleaved plasmid DNA isolated from derivatives of RB791 containing pACYC184 (to control for variations in plasmid yield during extraction), the satellite plasmid pMS316, and pMS92 (lanes a and b), pUT459 (lanes c and d), or pMS94 (lanes e and f). Cells were cultured in the presence or absence of 1 mM IPTG (isopropyl-β-D-thiogalactopyranoside) for six to seven generations before isolation of the plasmid DNA.

fragment containing *oriT* and derived from RSF1010 was reported by Willetts and Wilkins (36); their sequence is identical to ours except for position 139, where the RSF1010 sequence contains a G · C instead of an A · T base pair. We observed G · C at this position within the DNA cloned in pMS170, whereas A · T was found for all other cloned fragments. Because pMS170 contains a fully active *oriT*, this base change does not seem to be functionally important.

Although the *Taq*I fragment (5.53 to 5.69 kbp) includes an active *oriT*, the transfer frequency for the pBR322 derivative containing this DNA (pUT256) was only 1% of the frequency for the derivative containing the *Hae*III fragment (5.52 to 5.68 kbp) (Table 2). In addition, pUT333 (5.54 to 6.5 kbp) is *oriT*$^-$ (data not shown). We believe, therefore, that the 20 base pairs of DNA between 5.52 and 5.54 kbp are particularly important for an active *oriT*. The sequence of this DNA shows strong twofold symmetry, with a 10-base-pair inverted repeat (Fig. 2) that could serve as a recognition site for a multimeric Mob protein. The plasmid pUT256 might be poorly mobilized because it contains only half of this site. In any case, it is clear that in determining which base pairs make up *oriT*, it will be necessary to distinguish those required for transfer above the level of background from those enabling frequencies of transfer approaching that of the parental plasmid.

Region II, which is essential for mobilization, is located just to the left of *oriT*, with an endpoint between 5.38 and 5.48 kbp (Fig. 1). The results of experiments in which a *lacZ* gene fragment was fused to R1162 DNA at 5.1 kbp (unpublished result) suggest that transcription of region II is from right to left. There are two potential ATG initiation codons for right-to-left translation between 5.41 and 5.48 kbp, at base pairs 32 and 67 (Fig. 2), each associated with a potential ribosome binding site, 5′-GAGG (31), just upstream from the translation start. If both initiation codons are used, then there are two out-of-phase open reading frames in MobII. One of these could be responsible for the activity of MobIV. There is no obvious promoter sequence, conforming closely to known consensus sequences for procaryotic promoters, associated with these potential reading frames. This is also the case for the RepI region, in which protein products have been identified (26).

Region I is located within 5.54 and 6.1 kbp, and this entire segment has been sequenced (Fig. 2). There are several open reading frames in this DNA which could encode small proteins consisting of between 100 and 150 amino acids. We have no evidence that these proteins are made; experiments to identify the products encoded within each of the Mob regions are now underway.

TABLE 3. Summary of mobilization frequencies for plasmids containing different regions of Mob DNA

| Plasmid containing Regions[a]: | | | | | Mobilization frequency[b] |
|---|---|---|---|---|---|
| MobI | MobII | MobIII | MobIV | *oriT* | |
| + | + | + | + | + | $10^{-1}$–$10^{-2}$ |
| + | + | + | − | + | $10^{-3}$ |
| + | + | − | − | + | $10^{-5}$–$10^{-6}$ |
| + | − | − | − | + | $<10^{-8}$ |
| − | − | − | − | + | $<10^{-8}$ |
| − | + | + | + | + | $10^{-4}$–$10^{-5}$ |
| − | + | + | − | + | $10^{-6}$–$10^{-8}$ |
| − | + | − | − | + | $<10^{-8}$ |
| + | − | + | − | + | $<10^{-8}$ |

[a] +, Present; −, absent.
[b] Number of transconjugants per donor cell.

ACKNOWLEDGMENT

This research was supported by a grant from the National Science Foundation (PCM-8211916).

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