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Translocation of a Discrete Piece of Deoxyribonucleic Acid Carrying an *amp* Gene Between Replicons in *Escherichia coli*

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A number of well-characterized R plasmids (R1*drd*19.K1, R100-1, R46, R55-1, R64-11, R388, and R751) will mobilize an integrated *amp* gene from the chromosome of *Escherichia coli* K-12. R391 will not act in this way. The process involves recombination, and in each case the mobilizing plasmid acquires an additional piece of deoxyribonucleic acid of molecular weight 4×10^6. Acquisition of this deoxyribonucleic acid may impair the transfer properties of the recombinant plasmid in some cases. The process will occur in a *recA* background.

RP1-1 is a self-transmissible element which confers resistance to penicillins by specifying the synthesis of type IIIa β-lactamase (8, 11). It transfers freely between strains of *Pseudomonas aeruginosa* (20), but mating with *Escherichia coli* gives rise to colonies in which the β-lactamase gene is unstable (18). Prolonged subculture of such unstable clones on selective agar results in the appearance of a few colonies in which this gene is stable. Certain *E. coli* lines in which this process has occurred have been examined, and the gene in question has been found to be integrated into the bacterial chromosome (18).

The isolation of *E. coli* strains carrying an integrated *amp* gene derived from RP1-1 has allowed us to examine the translocation of this resistance determinant to a number of replicons, and this paper describes its translocation to several R plasmids. These experiments were made possible by two circumstances: first, the integration of the *amp* gene in *E. coli* is associated with a loss of self-transmissibility, and, secondly, this gene can be mobilized from its chromosomal site by a variety of R plasmids. This latter process often involves recombination of the *amp* gene with the mobilizing plasmid, a step that adds a piece of deoxyribonucleic acid (DNA) of molecular weight about 4×10^6. Moreover this process can occur in *E. coli recA*. The added piece of DNA seems to be a translocatable segment containing the *amp* gene originally present on RP1-1.

A preliminary account of this work has already appeared (P. M. Bennett, Proc. Soc. Gen. Microbiol., 1975).

MATERIALS AND METHODS

Origin of bacterial strains and plasmids. The bacterial strains and plasmids used in these experiments are listed, together with their sources, in Tables 1 and 2. All *recA* strains were tested to confirm their recombination deficiency with respect to chromosomal markers. In all cases the recombination frequencies obtained were at least 10,000-fold lower than in *rec*$^+$. *E. coli* UB1731 was isolated by a two-stage process. First, *P. aeruginosa* Ps18 (RP1-1) was mated with *E. coli* JC3272 and the exconjugant colonies were selected on nutrient agar containing 200 μg of streptomycin and 500 μg of carbenicillin per ml. A single colony picked from these plates showed the unstable phenotype typical of RP1-1 after transfer to *E. coli* (11, 18). A culture grown from this unstable colony was then mated with *E. coli* UB281 for 7 h, and the exconjugants were selected on minimal medium supplemented with proline, methionine, glucose, and 500 μg of carbenicillin per ml. Once again the exconjugants showed the instability characteristic of RP1-1 in *E. coli*. A single colony was again picked which, after two cycles of growth and reisolation on agar containing 500 μg of carbenicillin per ml, gave a single colony in which the expression of the β-lactamase gene was stable. Examination of this strain (UB1731) showed that the *amp* gene had become integrated somewhere between *trp* and *his* on the chromosome (P. M. Bennett, unpublished data).

Gene transfer by mating. Cultures of donor and recipient bacteria grown overnight in nutrient broth at 37 C were diluted 100-fold into fresh prewarmed nutrient broth and incubated with shaking at 37 C until the culture density of both had reached about 2×10^8 bacteria/ml. A sample of the donor culture (0.5 ml) was then mixed with 1.0 ml of recipient, and the mixture was incubated at 37 C with gentle agitation either for 30 min or 2 h, as required. Dilutions of this mixture were then plated on appropriate selective agar.

Several R$^-$ recipient strains have been used in these experiments, all of them auxotrophic. Since all the donors were multiple auxotrophs, selection was carried out on minimal agar supplemented with an appropriate antibiotic to select for resistance transfer and the necessary amino acid or thymine

supplementation to allow growth of those recipient bacteria that had received the resistance marker. The nutritional requirements of the *E. coli* strains are shown in Table 1.

The following antibiotics have been used for selection as appropriate (concentrations in micrograms per milliliter): carbenicillin, 500; kanamycin, 30; spectinomycin, 50; tetracycline, 10. Trimethoprim was used in most experiments at a concentration of 20 μg/ml. With strain UB1139 as recipient the concentration needed was 1 mg/ml.

Tests for plasmid incompatibility were carried out by studying the ability of two plasmids to coexist stably in the absence of selection pressure for markers on either plasmid.

Isolation of plasmid DNA. All plasmids were transferred by standard mating procedures to the *E. coli* thymine auxotroph UB1139 for extraction of plasmid DNA (8). Cultures of UB1139 carrying the appropriate plasmid were grown overnight at 37 C in minimal medium supplemented with 1 μg of thymine per ml. In the morning these cultures were diluted 20-fold with fresh medium containing 1 μg of nonradioactive and 5 μCi of [^{3}H]thymine per ml. When the culture density had reached approximately 5×10^8 organisms/ml, the bacteria were collected, washed, and then lysed as described previously (8). Plasmid DNA was isolated as covalently closed, circular DNA on ethidium bromide-CsCl gradients (17). The fractions containing the denser satellite band were pooled and cleared of ethidium bromide by extraction with isopropanol saturated with CsCl and then dialyzed against 0.15 M ammonium acetate (15). Solutions prepared in this way were stored at −20 C until required for use.

Estimation of the contour length of plasmids. DNA/cytochrome *c* films were prepared and transferred to electron microscope grids as described by Lang and Mitani (15). Plasmid DNA was visualized by rotary shadowing with platinum at an angle of 10.5° at a distance of 10 cm, and the grids were examined in a Hitachi HS-7S electron microscope. Unambiguously open circular molecules were photographed and printed on Kodalith LP photographic paper (Kodak, Ltd., London), and contour lengths were measured with a map measurer. Magnification factors were calculated using Dow latex beads (Dow Chemical Co.) of 1.099-μm diameter.

Approximate molecular weights were calculated from the contour lengths by using a conversion factor of 1 μm = 2.07×10^6 (14).

TABLE 1. *E. coli K-12 strains used in these experiments*

Strain no.	Relevant genetic markers	Origin	Reference
UB281	*pro⁻ met⁻ nal^r*	Spontaneous *nal^r* mutant isolated from J53	
UB1731	*pro⁻ met⁻ nal^r amp^r*	Derived from UB281	
UB1780	*pro⁻ met⁻ nal^r amp^r recA56*	Derived from UB1731	
JC3272	*trp⁻ his⁻ lys⁻ str^r*	A. J. Clark	1
JC6310	*trp⁻ his⁻ lys⁻ str^r recA56*	Derived from JC3272	1
UB1139	*leu⁻ thy⁻ met⁻ mal^r*	Derived from UB1005	8

RESULTS

Transfer of the amp gene from strain UB1731 using R plasmids. Attempts to transfer the *amp* gene from *E. coli* UB1731 to R⁻ strains of *E. coli* have always failed (limit of detection, 10^{-9}/donor). However, a number of R plasmids can mobilize this gene from the chromosome. In a typical example, UB1731 carrying R388 was mated with *E. coli* JC 6310 in a 2-h cross, and the progeny was selected as described above. A total of 100 carbenicillin-resistant colonies were obtained, and all of these were also resistant to trimethoprim when replica plated (Table 3). In the absence of R388 no transfer of *amp* was obtained. R388 therefore allows transfer of the *amp* gene from UB1731 at a frequency of about one-hundredth the rate found for the transfer of R388 alone. As there is 100% linkage between the penicillin and trimethoprim resistance genes when the initial selection takes place on carbenicillin, the mobilization of the *amp* gene from the chromosome

TABLE 2. *R plasmids used in these experiments*[a]

Plasmid no.	Incompatibility	Resistance pattern	Supplied by:	Reference
R388	W	Tp Su	N. Datta (RPGMS)	5
R751	P	Tp	N. Datta (RPGMS)	12
R46	N	Amp Sm Sp Tc Su	J. T. Smith (SP)	7
R100-1	FII	Sm Su Cm Tc	This laboratory	
R1*drd*19.K1	FII	Kn	This laboratory	2
R391	J	Kn	D. Bouanchaud (IP)	4
R55-1	C	Cm Su	D. Bouanchaud (IP)	21
R64-11	I	Tc Sm	N. Datta (RPGMS)	6

[a] Abbreviations: RPGMS, Royal Postgraduate Medical School, Hammersmith, London; SP, School of Pharmacy, London University, London; IP, Institut Pasteur, Paris; Tp, trimethoprim; Su, sulphonamide; Amp, ampicillin; Sm, streptomycin; Sp, spectinomycin; Tc, tetracycline; Kn, kanamycin; Cm, chloramphenicol.

TABLE 3. *Transfer frequency of amp^r from UB1731 into JC6310*

R factor mediating transfer	Transfer frequency[a] (Amp^r transcipients/R$^+$ transcipients)
R388	1.3×10^{-2}
R751	2.0×10^{-2}
R46	4.2×10^{-4}
R100-1	2.0×10^{-6}
R1*drd*19.K1	2.4×10^{-3}
R391	Undetected
R55-1	1.0×10^{-2}
R64-11	7.1×10^{-4}

[a] Genetic transfer was performed as described in Materials and Methods using a 2-h mating period prior to plating on selective agar.

of UB1731 may involve recombination between the plasmid and the *amp* gene.

This conclusion is supported by experiments in which progeny colonies from this cross were used in a further transfer experiment with an R$^-$ recipient (UB281). Such donors transferred the recombinant R plasmid at a frequency of about 10^{-1}/donor when selection was for trimethoprim resistance (Table 5). All the trimethoprim-resistant colonies obtained were also pencillin resistant.

Similar experiments have shown that R46, R751, R100-1, R144, R1-19.K1, R64-11, and R55-1 can all mobilize the *amp* gene from strain UB1731. The only plasmid that failed to mobilize *amp* was R391. R388 showed the greatest frequency of transfer and R100-1 showed the smallest when compared with frequency of R-plasmid transfer in the same cross (Table 3). In all cases examination of the progeny of the cross showed that exconjugants that had received *amp* had also received the relevant plasmid markers and that outcrossing of these exconjugant colonies to R$^-$ recipients always showed 100% linkage between the *amp* gene and the markers of the plasmid involved.

Isolation of plasmid DNA after mobilization of the amp gene. These genetic experiments suggest that mobilization of the *amp* gene from the chromosome of strain UB1731 with a plasmid may commonly involve recombination with the mobilizing element. To decide whether this had occurred, plasmids that had succeeded in mobilizing *amp* from *E. coli* UB1731 were isolated as covalently closed, circular DNA, and their molecular characteristics were compared with those of the parent plasmid used initially to achieve mobilization. To facilitate this study, all the plasmids concerned were transferred to UB1139, an *E. coli* strain already known to be free of covalently closed, circular DNA and to take up [^{3}H]thymine efficiently. The transfers were all carried out for 2 h under standard mating conditions, and plasmid transfer was selected for on appropriate agar. In all cases transfer of the plasmid resistance marker was accompanied by the linked transfer of penicillin resistance (data not shown).

The molecular properties of the plasmids involved in these experiments are shown in Table 4. In all cases plasmids that had mobilized the *amp* gene from UB1731 had acquired an additional piece of DNA of molecular weight about 4.0×10^6 when compared with the original plasmid.

Properties of the plasmid Amp recombinant elements. Recombination between chromosomal and plasmid DNA occurred in the formation of these plasmids, and this raises the question of whether the process had modified any of the plasmid characters. For example, are the incompatibility relationships and the ability of the plasmid to achieve entry exclusion altered and is the expression of the resistance determinants changed?

To detect any effects on entry exclusion, strains carrying plasmids into which the *amp* gene had been inserted were tested for their ability to exclude related elements. For example, *E. coli* JC6310 carrying the ampicillin-resistant derivative of R751 was used as recipient, and the frequency of transfer of RP1 to this strain was compared with the frequency of transfer of the same plasmid to an otherwise isogenic R$^-$ recipient. The mating experiments were carried out under standard conditions for 2 h, and the entry of the incoming plasmid marker was detected on appropriate antibiotic-containing agar. Whereas the plasmid RP1 was

TABLE 4. *Sizes of parent R plasmids and amp^r derivatives*

R plasmid	Contour length[a] (μm)	Mol wt[b] ($\times 10^6$)	Mol wt of DNA added to parent R plasmids ($\times 10^6$)
R388	10.4 ± 0.1	21.5	
R388/*amp*	12.3 ± 0.2	25.5	4
R46	16.1 ± 0.2	33.3	
R46/*amp*	18.0 ± 0.3	37.3	4
R751	16.8 ± 0.2	34.9	
R751/*amp*	18.7 ± 0.3	38.9	4
R1*drd*19.K1	22.9 ± 0.3	47.4	
R1*drd*19.K1/*amp*	24.9 ± 0.2	51.5	4.1

[a] Values are the average of measurements of 10 or more individual molecules of each R plasmid.

[b] Molecular weight was calculated from contour length using a conversion factor of 1 μm = 2.07×10^6 (14).

found to transfer to the R⁻ recipient at a frequency of about 10^{-1}/donor, transfer to the recipient carrying the R751/*amp* recombinant element was reduced by a factor of almost 1,000-fold. Reductions of between 100- and 1,000-fold were also obtained with all the other plasmid/*amp* recombinant elements, and in no case is there any evidence that recombination with the *amp* gene has altered the entry exclusion potential of any of the R plasmids involved. Similarly, there is no evidence that insertion of the *amp* gene into any of the R plasmids used in these studies alters their incompatibility properties.

In no case studied here has the acquisition of the *amp* gene led to changes in the other resistance characters carried by the plasmids concerned, and moreover this applies both to the nature and level of resistance expressed.

Transfer of R plasmid/Amp recombinants. Another property of the R plasmids that may have been altered by their recombination with the *amp* gene in the course of mobilization is the ability of the recombinant plasmid to promote its own transfer to R⁻ recipients. To examine this point, *E. coli* strain JC6310 carrying an R plasmid/Amp recombinant element was mated in a standard 30-min cross with *E. coli* UB281, and the progeny was selected on appropriate agar. Transfer of the parental plasmid from the same host strain to UB281 was also measured as a control. Recombination of the *amp* gene with the plasmid DNA had no effect on the frequency of transfer of any of the plasmids save R388 (Table 5). With this plasmid the results were less clear-cut. In all, six clones carrying the R388/Amp recombinant plasmid were tested. Three of these showed the same frequency of transfer to strain UB281 as R388 itself, two transferred at a markedly lower frequency, and in one case transfer of the recombinant plasmid could not be detected, regardless of whether transfer was selected for with carbenicillin or with trimethoprim. In the majority of cases, therefore, recombination of the *amp* gene with the mobilizing plasmid has no effect on the transfer potential of the plasmid.

Role of the host recombination system in the formation of R plasmid/Amp recombinants. All the R plasmids used to mobilize the *amp* gene from *E. coli* UB1731, save R391, transferred the *amp* gene with similar frequencies from the *recA* mutant UB1780. As mentioned previously R391 cannot mobilize the *amp* gene at all, even from a *rec*⁺ donor. The effectiveness in this process depends on the nature of the plasmid involved. Mobilization from UB1780 with R388 occurs at the same frequency as mobilization in UB1731. On the other hand, R46 was about 30-fold more effective in a *rec*⁺ than in a *recA* background. However, in no case was the ability of a plasmid to mobilize the *amp* gene from the chromosome reduced in *recA* hosts to the extent that chromosomal recombination of metabolic markers was impaired in the same strain. The results of these experiments are summarized in Table 6.

In no case could any of the plasmids examined here mobilize chromosomal markers (*trp*, *his*) from either UB1731 or UB1780 into JC6310.

TABLE 5. *Transfer frequencies of parent plasmids and ampʳ derivatives from JC6310 into UB281*

R plasmid	Transfer frequency[a] (R⁺ transcipients/donor)
R388	1.8×10^{-1}
R388/*amp1*	8.3×10^{-2}
R388/*amp2*	1.1×10^{-1}
R388/*amp3*	No transfer detected[b]
R388/*amp4*	2.4×10^{-1}
R388/*amp5*	No transfer detected
R388/*amp6*	No transfer detected
R46	6.4×10^{-2}
R46/*amp1*	7.3×10^{-2}
R46//*amp2*	7.1×10^{-2}
R751	1.4×10^{-1}
R751/*amp1*	2.2×10^{-1}
R751/*amp2*	1.7×10^{-1}
R1*drd*19.K1	1
R1*drd*19.K1/*amp1*	1
R1*drd*19.K1/*amp2*	1

[a] Genetic transfer was performed as described in Materials and Methods using a 30-min mating period prior to plating on selective media.

[b] Transfer was detectable after overnight mating with R388/*amp3* and R388/*amp6*.

TABLE 6. *Transfer frequency of ampʳ from UB1780 into JC6310*

R plasmid mediating transfer	Transfer frequency[a] (Ampʳ transcipients/R⁺ transcipients)
R388	1×10^{-2}
R751	2×10^{-3}
R46	1.7×10^{-5}
R100-1	5.2×10^{-7}
R1*drd*19.K1	7.9×10^{-3}
R391	Undetected
R55-1	8×10^{-5}
R64-11	1×10^{-3}

[a] Genetic transfer was performed as described in Materials and Methods using a 2-h mating period prior to plating on selective agar.

DISCUSSION

The mobilization of the *amp* gene from its chromosomal site in *E. coli* UB1731 can be

achieved by a number of well-characterized R plasmids. The process always involves recombination, and the molecular weight of the plasmid concerned is always increased by a molecular weight of 4×10^6 as a result. Not all R plasmids seem to mobilize in this way. For example, neither we nor Hedges and Jacob (9) could obtain recombinants involving the *amp* gene and R391.

When a piece of DNA is integrated into an R plasmid, there is the chance that the event may modify the properties of the recipient element. With most of the plasmids used in these studies (e.g., R46, R751, R1-19K1) no differences in the properties of the recombinant elements were detected beyond the acquisition of the penicillinase determinant and an increase in size. However, the number of recombinant elements examined in detail is still small. With R388, however, recombination produced a number of different types of recombinant plasmid, two being altered in transfer function and one being unable to transfer at all (see Table 4). This suggests that the insertion of the *amp* gene into R388 does not occur at a single site in all plasmids. Similar results have been reported by Hedges and Jacob (9) after insertion of the *amp* gene from RP4 into the W-group plasmid Sa.

The detection of this translocation unit involving the *amp* gene in the chromosome of UB1731 throws some light on the origin of some of the strains used in these experiments. Strain UB1731 was constructed by mating *P. aeruginosa* 1822 (RP1-1) with *E. coli* R^- (see Materials and Methods). The chromosomal integration of the *amp* gene in this recipient and its subsequent translocation to other plasmids imply that the same translocation unit containing the *amp* gene is probably also present in RP1-1.

The element RP1-1 has never been detected as covalently closed, circular DNA in either *P. aeruginosa* or *E. coli* (2). It was originally detected in strains of *P. aeruginosa* that had originally harbored RP1, a P class plasmid specifying resistance to neomycin, kanamycin, and tetracycline as well as to penicillins (18), after that element had been lost spontaneously (11). Originally it was thought that RP1-1 was a fragment of RP1 retaining only the penicillinase gene and some transfer functions (11). Now, however, the ready translocation of the *amp* gene that occurs both from RP4 (alias RP1) and from RP1-1 suggests that the latter element may have arisen in *P. aeruginosa* 1822 by translocation of the *amp* gene from RP1 to some other element present in that strain but undetected. This possibility is supported by the clear differences in phage interaction and plasmid incompatibility now known to exist between RP1 and RP1-1 (3, 13, 19; T. Morgan and V. Stanisich, J. Gen. Virol., in press) and also by the detailed properties of the β-lactamase protein specified by the *amp* gene from these two elements (16). So far this minor enzyme variant has only been detected in bacteria carrying either RP1 or RP1-1.

The fact that the translocation unit studied here may be the same as the one present on RP1 argues that this element is related to "transposon A" described by Hedges and Jacob (9). The molecular weight of the unit inferred from the molecular data reported here is similar to that given by Falkow and his collaborators (10), although the size determined by us is more uniform. The most clear-cut difference between our observations and those published elsewhere (9, 10) concerns the requirement for a functional *recA* gene product for translocation of the *amp* gene to occur. The work described here shows the process to occur readily in a tight *recA* background.

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