

## SHORT PAPER

# Transposon-Directed Mutagenesis and Chromosome Mobilization in *Acinetobacter calcoaceticus* EBF65/65

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### SUMMARY

RP4::Mu was used to insert Tn3171 into the *Acinetobacter calcoaceticus* EBF65/65 chromosome. Insertions occurred at a frequency of approximately  $10^{-7}$  per recipient cell. Auxotrophic mutations resulted from 28% of the Tn3171 insertions examined, with a possible 'hot-spot' for insertion in a gene for arginine biosynthesis. The frequency of subsequent precise excision of Tn3171 from the chromosome was less than  $10^{-10}$ . When attempts were made using RP4 derivatives to mobilize the chromosome from a strain containing an *arg*::Tn3171 insertion, it was found that the enhanced transfer frequency of the adjacent region of the chromosome was dependent on the original Tn3171 insertion, but was independent of the presence of Tn3171 on the mobilizing plasmid.

### 1. INTRODUCTION

Plasmid RP4 has been shown to mobilize the chromosome of *Acinetobacter calcoaceticus* EBF65/65 at a low frequency from one of at least two alternative origins (Towner & Vivian, 1976*a, b*). Using this system of gene transfer, a circular linkage map has been established (Towner, 1978); however, transfer of markers distal to the donor origins of RP4-mediated chromosome transfer was particularly low, making it difficult to resolve the order of chromosomal markers in certain regions of the map.

RP4 and its relatives are known to be capable of mobilizing the chromosomes of a variety of Gram-negative bacteria at a low frequency (Holloway, 1979). In *Escherichia coli* K12 it is thought that this mobilization may occur as the result of an integration event following the transposition of a Tn1 copy from RP4 to the host cell chromosome (Harayama, Tsuda & Iino, 1980). Although Tn1 can transpose readily between plasmids, chromosomal insertions can only be obtained at a very low frequency at a restricted number of sites (Sherratt, 1981) and this could account for the low frequency of chromosome mobilization observed. Other transposons insert into the *E. coli* K12 chromosome at a number of different sites at a higher frequency (Sherratt, 1981). A possible method for the generation of chromosome mobilization from particular desired sites would therefore be to select for the insertion of a transposon (other than Tn1) into a gene of interest located on the chromosome, followed by mobilization from the insertion site using an identical transposon on the mobilizing plasmid. This paper describes the application of this method to *A. calcoaceticus* EBF65/65.

## 2. MATERIALS AND METHODS

Bacterial strains and plasmids are listed in Table 1. *Acinetobacter* minimal medium (AMM), other media and mating techniques were as described by Towner & Vivian (1976*a, b*). All matings were carried out at 30 °C. Where necessary, antibiotics were added to selective media at the concentrations used by Towner & Vivian (1977), with the exception of trimethoprim lactate which was used at a concentration of 200 µg/ml. Cultures of *E. coli* and *A. calcoaceticus* were tested for the production of bacteriophage Mu as described by Towner (1980).

Table 1. *Bacterial strains and plasmids*

		Reference
1. Strains of <i>E. coli</i> :		
TM362	clinical isolate carrying Tn3171	} Towner (1981)
K12 J53.2	F <sup>-</sup> <i>pro met rif</i>	
K12 CSH52	F <sup>-</sup> <i>pro thi lac ara strA recA</i>	
2. Strains of <i>A. calcoaceticus</i> EBF65/65		
C48	<i>ile-1 met-1</i>	} Towner (1978)
C426	<i>trp-2 his-1</i>	
C4508	<i>trp-2 his-1 arg::Tn3171</i>	This paper
3. Plasmids:		
RP4	Ap Km Tc	} Towner (1980)
pGV5001	RP4::Mu <i>cts61</i>	
pUN308	pGV5001::Tn3171	This paper
pUN153a	RP4 Ap::Tn3171	Towner (1981) and this paper

## 3. RESULTS

(i) *Construction of a vector for transposon insertions*

Towner (1980) demonstrated that plasmids carrying the lysogenic bacteriophage Mu have great difficulty in becoming established in *A. calcoaceticus* EBF65/65. As a first step towards selecting chromosomal transposon insertions it was therefore decided to construct an RP::Mu vector carrying a suitable transposon. *E. coli* TM362 carries the trimethoprim/streptomycin transposon Tn3171 on its chromosome (Towner, 1981). The RP4::Mu derivative pGV5001 was introduced into TM362 and a resulting transconjugant crossed with *E. coli* K12 J53.2, selecting for Tp<sup>r</sup> mobilization. This occurred at a frequency of approximately 10<sup>-6</sup> per recipient cell, compared with a normal pGV5001 transfer frequency of 10<sup>-2</sup> per recipient cell. When the Tp<sup>r</sup> transconjugants were tested for the continued production of viable Mu particles, only 15/50 transconjugants tested were still Mu<sup>+</sup>.

Twelve Tp<sup>r</sup> Mu<sup>+</sup> transconjugants were purified and crossed with the *recA* strain CSH52. When the frequencies of transfer of Tp<sup>r</sup> and ampicillin resistance (a pGV5001 marker) were compared, it was found that for each of the twelve transconjugants tested, Tp<sup>r</sup> was now transferred at the same frequency as Ap<sup>r</sup>, demonstrating that Tn3171 had transposed to pGV5001. One such pGV5001 derivative was selected for use as the vector in this study and designated pUN308 (= RP4::Mu*cts61*::Tn3171).

(ii) Selection of transposon insertions in the *A. calcoaceticus* chromosome

*E. coli* J53.2 (pUN308) was crossed with *A. calcoaceticus* C426. When selection was made on tetracycline-containing medium, no transconjugants were detected ( $< 10^{-9}$  per recipient cell), but when selection was made on Tp-containing medium, Tp<sup>r</sup> colonies were obtained at a frequency of  $10^{-7}$  per recipient cell. 50 of these colonies were purified and tested for Tc<sup>r</sup> and production of Mu particles. All were Tc<sup>s</sup> and failed to produce viable Mu particles, indicating that Tn3171 had transposed either to the chromosome or to some other plasmid present in C426 (Hinchliffe, Nugent & Vivian, 1980), while the pGV5001 component of pUN308 had been eliminated (Towner, 1980).

A further 100 Tp<sup>r</sup> transconjugants were picked off and patched on the same medium. Ten of these patches failed to grow and possibly represented examples of unstable Tn3171 insertions. The remaining 90 patches were replica-plated to AMM + *trp* + *his* + Tp (*trp* and *his* being the requirements of the parental C426). Of 90 patches 28 failed to grow on this medium (ie. had acquired an additional growth requirement). Twenty-one of these 28 strains were found to require *arg* + *trp* + *his*; 3 strains required *ile* + *trp* + *his*, while 4 strains failed to grow on any of the combinations tested, indicating the acquisition of a multiple requirement.

One of the isolates in which Tn3171 had apparently inserted into a gene governing arginine biosynthesis was selected for further study and designated C4508. To test the stability of C4508, an overnight culture of C4508 (about  $2 \times 10^{10}$  cells) was centrifuged, the cells resuspended in quarter-strength Ringer's solution and 0.1 ml portions spread on plates of AMM + *trp* + *his*. Following incubation, a single colony was obtained which was found, on testing, to have lost the requirement for arginine and, simultaneously, to have lost the resistance to trimethoprim specified by Tn3171. This demonstrated that the insertion of Tn3171 was directly responsible for the acquisition of the additional auxotrophic requirement and that the frequency of subsequent precise excision of Tn3171 from C4508 was less than  $10^{-10}$ .

## (iii) Chromosomal location of the Tn3171 insertion in C4508

C48 (RP4) was crossed with C4508 and recombinants selected and analysed using co-inheritance frequencies (Towner, 1978). C48 (RP4) donates its chromosome primarily from the DO donor site (Towner & Vivian, 1976*b*) and the results obtained (Table 2) were consistent with the *arg* marker (and hence the Tn3171 insertion) being located very close to the distal *ile* marker, as shown in Fig. 1.

## (iv) Chromosome mobilization using RP4::Tn3171

pUN153a is a derivative of RP4 in which Tn3171 is inserted into the Ap<sup>r</sup> gene (part of Tn1) on RP4. pUN153a was transferred to C4508 and a resulting transconjugant crossed with C48 in parallel with crosses in which C426 (RP4), C426 (pUN153a) and C4508 (RP4) were used as donors. The results obtained (Table 3) show that the *ile*<sup>+</sup> marker was transferred at an approximately 10-fold increased frequency when either C4508 (RP4) or C4508 (pUN153a) were used as the donors. This result indicated that the enhanced transfer frequency of the *ile*<sup>+</sup> marker was a consequence of the Tn3171 insertion in C4508, but was independent of the initial presence of Tn3171 on the mobilizing plasmid. A corresponding increase was not detected for transfer of the *met*<sup>+</sup> marker, indicating that the enhanced frequency of mobilization of *ile*<sup>+</sup> was perhaps a consequence of unidirectional transfer from a site located between *met* and *ile*. This would be consistent

Table 2. *Co-inheritance frequencies of arg::Tn3171 with other markers*

Cross: C48(RP4) × C4508

Selected marker	Unselected marker	Co-inheritance (%)
<i>his</i> <sup>+</sup>	<i>trp</i> <sup>+</sup>	30.6
	<i>arg</i> <sup>+</sup>	0
	<i>met</i>	0
	<i>ile</i>	0
<i>trp</i> <sup>+</sup>	<i>his</i> <sup>+</sup>	78.0
	<i>arg</i> <sup>+</sup>	0
	<i>met</i>	3.0
	<i>ile</i>	0
<i>arg</i> <sup>+</sup>	<i>his</i> <sup>+</sup>	46.6
	<i>trp</i> <sup>+</sup>	43.6
	<i>met</i>	42.3
	<i>ile</i>	100

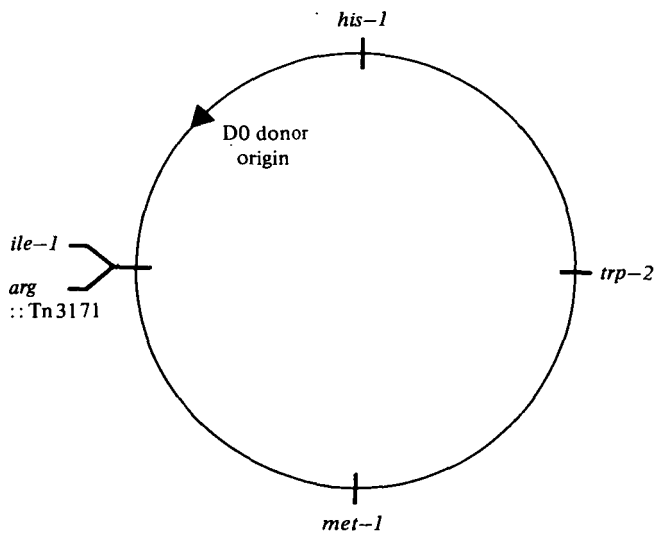


Fig. 1. Chromosomal location of the Tn3171 insertion in C4508.  
Markers are arbitrarily spaced.

with mobilization from the Tn3171 insertion in *arg*, which mapped very close to the *ile* locus. When 100 *ile*<sup>+</sup> recombinants were tested for co-inheritance of the appropriate unselected markers, although it was apparent that large segments of chromosome were being transferred, no asymmetry was detectable in the inheritance of the unselected markers and it was not possible to draw any firm conclusions about the direction and origin of chromosome transfer. It therefore seemed possible that chromosome transfer from C4508 (pUN153a) was occurring from more than one site in more than one direction.

Table 3. Relative frequencies of different recombinant classes

Cross		Marker selected from donor	Average no. of recombinants /10 <sup>8</sup> recipient cells
Donor	Recipient		
C426(RP4)	C48	<i>ile</i> <sup>+</sup>	12
C426(pUN153a)	C48	<i>ile</i> <sup>+</sup>	10
C4508(RP4)	C48	<i>ile</i> <sup>+</sup>	100
C4508(pUN153a)	C48	<i>ile</i> <sup>+</sup>	126
C426(RP4)	C48	<i>met</i> <sup>+</sup>	2
C426(pUN153a)	C48	<i>met</i> <sup>+</sup>	3
C4508(RP4)	C48	<i>met</i> <sup>+</sup>	1
C4508(pUN153a)	C48	<i>met</i> <sup>+</sup>	1

## 4. DISCUSSION

The results presented in this paper demonstrate that a P incompatibility group plasmid carrying bacteriophage Mu can function as an efficient vector for the insertion of a transposon (Tn3171) into the *A. calcoaceticus* EBF65/65 chromosome. It seems probable that Tn3171 alone was transposed, but the possibility that some remaining portion of the vector plasmid was also simultaneously inserted could not be entirely ruled out.

It has been reported that the prototype TpSm transposon Tn7 has a unique insertion site in the *E. coli* chromosome, but it is not yet known whether the high efficiency with which Tn7 transposes into other organisms such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Agrobacterium tumefaciens* involves a similar site (Lichtenstein & Brenner, 1982). In *A. calcoaceticus* EBF65/65 it seems that the related transposon Tn3171 has several possible insertion sites, but with a possible 'hot-spot' for insertion in a gene governing arginine biosynthesis. It appears unlikely that Tn3171 would be useful as a generalized mutagen in EBF65/65; however, other transposons with an even lower specificity of insertion may be useful in this respect.

When attempts were made to mobilize the chromosome from a strain containing a Tn3171 insertion, it was found that enhanced frequencies of transfer of particular regions of the chromosome were dependent on the original chromosomal Tn3171 insertion, but were independent of the presence of Tn3171 on the mobilizing plasmid. One possible explanation would be that the majority of RP4-generated recombinants resulted from the prior relatively high frequency transposition of Tn3171 to RP4 so that effectively, as far as chromosome mobilization of *ile* was concerned, RP4 and pUN153a were the same.

The chromosome mobilization results demonstrated that it was possible to influence the pattern of chromosome mobilization by the prior insertion of a transposon into the EBF65/65 chromosome. This is the first direct evidence of the involvement of transposition in RP4-generated chromosome mobilization in *A. calcoaceticus*. The technique described may prove to be a useful method for enhancing the transfer frequencies of particular regions of the chromosome in this organism.

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