INSERTIONS OF THE TRANSPOSON Tn*1* INTO THE *PSEUDOMONAS AERUGINOSA* CHROMOSOME

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ABSTRACT

The transposon Tn*1* has been translocated to the chromosome of *Pseudomonas aeruginosa* from plasmid R18, following hydroxylamine mutagenesis of the plasmid. Twelve insertions were mapped to six distinct sites distal to 55 min of the origin of chromosome transfer by the plasmid FP2. These map locations were confirmed by host chromosome mobilization tests mediated by plasmids R18 or R91–5, due to Tn*1* homology between plasmid and host chromosome. All the Tn*1* chromosomal inserts were retransposable to other plasmids (Sa, R931 and R38). The behavior of Tn*1* in *P. aeruginosa* was very similar to its behavior in *Escherichia coli* with respect to regional specificity, orientation of insertion and in serving as regions of homology for host chromosome mobilization by plasmids. This last property has permitted the demonstration that Tn*1* on R18 and R91–5 is in opposite orientation with respect to the origin of transfer (*oriT*) of the two plasmids.

THE range of genetic manipulations possible with prokaryotic genomes has been enormously increased with the discovery of transposable genetic elements (KLECKNER, ROTH and BOTSTEIN 1977). In particular, the portable regions of homology between the same elements on a plasmid and the bacterial chromosome have facilitated the construction of Hfr donors with predetermined origins and directions of chromosome transfer, the creation of new F primes and the isolation of more efficient donors in *Vibrio cholerae*, (KLECKNER, REICHARDT and BOTSTEIN 1979; CHUMLEY, HENZEL and ROTH 1979; JOHNSON and ROMIG 1979). One of these elements, Tn*3*, coding for ampicillin resistance (Amp^r), has been sequenced and the requirements for its transpositional activity determined both from genetic and molecular data (HEFFRON *et al.* 1979; GILL, HEFFRON and FALKOW 1979; CHOU *et al.* 1979). For transposition, a 38-base-pair inverted repeat at the termini is absolutely required in *cis*, in addition to a transposase gene. The regulatory gene for the transposase and a site whose absence leads to dual insertion of the element as direct repeats in the recipient genome have also been identified. Tn*3* is part of a family of homologous transposons, TnA, that includes Tn*1* (CAMPBELL *et al.* 1979; HEFFRON *et al.* 1979). Tn*1* was the first transposon (element coding for antibiotic resistance in addition to its transposi-

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tional activity) discovered in prokaryotes. It was identified on the promiscuous plasmid RP4, which was initially isolated from *Pseudomonas aeruginosa* (HEDGES and JACOB 1974). From the same *P. aeruginosa* strain, we isolated the plasmid R18 (CHANDLER and KRISHNAPILLAI 1974b), which has subsequently been shown to be identical to RP4 by analysis of fragments obtained by single and double digests by a variety of restriction enzymes (STOKES, MOORE and KRISHNAPILLAI 1981).

In the course of studying the transfer genes of R18 in *P. aeruginosa*, we isolated transfer-deficient (Tra^-) mutants by *in vitro* mutagenesis with hydroxylamine of transducing phage lysates made on bacteria harboring R18, which were subsequently used to transduce *P. aeruginosa* recipients. Twelve transductants selected by carbenicillin resistance (Cb^r; coded by *Tn1*) turned out to be due to the transposition of Tn*1* onto the bacterial chromosome. This paper describes their genetic location, transpositional activity and usefulness as portable regions of homology for host chromosome mobilization in *P. aeruginosa*.

MATERIALS AND METHODS

Bacterial strains, plasmids and phages: These are shown in Table 1. The strains are all derivatives of strain PAO of *P. aeruginosa* (HOLLOWAY, KRISHNAPILLAI and MORGAN 1979). The plasmids FP2 and R68.45 (a Cb^s derivative of R68.45, designated pMO850, was used here) are extensively used for the chromosome mapping of strain PAO (HOLLOWAY, KRISHNAPILLAI and MORGAN 1979).

Media: Nutrient agar (NA) and nutrient broth (NB) have been described by KRISHNAPILLAI (1971). NB or NB supplemented with $NaNO_3$ (0.4% w/v) was used for routine culturing of bacteria. When antibiotics were used, their final concentration in NA was: streptomycin (SM), Sigma Chemical Company, 150 μg/ml; rifampin (RIF) as Rifadin, Lepetit Pharmaceuticals, Ltd., 150 μg/ml; neomycin (NM), Sigma, 300 μg/ml; tetracycline (TC), Sigma 100 μg/ml; chloramphenicol (CM), Sigma 300 μg/ml; carbenicillin (CB), Beecham Research Laboratories, 250 μg/ml. Mercuric chloride (HG), was 12 μg/ml in NA. Sulfanilamide (SU), British Drug Houses, Ltd., at a concentration of 150 μg/ml was used in lysed horse blood agar (KRISHNAPILLAI 1979). When antibiotics were used in minimal agar (MM), their concentration was SM, 250 μg/ml; CB, 500 μg/ml. MM was that of VOGEL and BONNER (1956). Minimal medium P (MP) was that of LEISINGER, HAAS and HEGARTY (1972). Amino acids and purines were added to MM or MP as required to a final concentration of 1 mM, except isoleucine, which was at 0.5 mM. Tyrosine, carnosine, mannitol, sodium benzoate and acetamide were used in MP as sole carbon sources at 0.1%, except for acetamide, which was used at 0.3%.

Phage methods: These were as described previously (KRISHNAPILLAI 1971).

Host chromosomal recombination via *conjugation or transduction and linkage analysis*: Plate mating conjugations utilized log-phase donors at *ca.* 5×10^8 cells/ml and stationary phase recipients at *ca.* 2×10^9 cells/ml. Equal volumes (0.1 ml) of donor (or dilutions) and recipient were spread on selective media. G101 transductions were performed as described by KRISHNAPILLAI (1971). Prototrophic recombinants were selected on MM or MM supplemented appropriately. Recombinants able to utilize carbon sources (*cnu*+ on carnosine, *mtu*+ on mannitol, *tyu*+ on tyrosine, *cat*+ on sodium benzoate, *hex*+ on glucose and *ami*+ on acetamide) were selected on MP or MP supplemented appropriately. Purified recombinants were scored for unselected markers by replica plating.

*Isolation of Cb^r transductants by E79*tv-2: A phage lysate prepared on PAO2(R18) was mutagenized *in vitro* with 2 M hydroxylamine for 24 hr at 37° (SCOTT 1968). The acetone-neutralized lysate was then used to transduce PAO2 by selecting for Cb^r transductants on NA + CB prespread with a 1/50 dilution of E79 anti-serum ($K = 400$/min). Transductants were repeatedly purified to free them of phage before further characterization.

TABLE 1

Bacterial strains, plasmids and phages used

Strain/plasmid/ phage	Genotype/phenotype	Source/reference
P. aeruginosa strains		
PAO1	*cml-2* prototroph FP2-	ISAAC and HOLLOWAY (1968)
PAO2	*ser-3* FP2-	ISAAC and HOLLOWAY (1968)
PAO25	*argF leu-10*	HAAS and HOLLOWAY (1976)
PAO429	*ser-3 bla-429* FP2-	This paper
PAO431	*ser-3 bla-431* FP2-	This paper
PAO432	*ser-3 bla-432* FP2-	This paper
PAO433	*ser-3 bla-433* FP2-	This paper
PAO434	*ser-3 bla-434* FP2-	This paper
PAO435	*ser-3 bla-435*FP2-	This paper
PAO436	*ser-3 bla-436* FP2-	This paper
PAO437	*ser-3 bla-437* FP2-	This paper
PAO438	*ser-3 bla-438* FP2-	This paper
PAO439	*ser-3 bla-439* FP2-	This paper
PAO440	*ser-3 bla-440* FP2-	This paper
PAO441	*ser-3 bla-441* FP2-	This paper
PAO505	*met-9011 ami-200* FP2-	D. HAAS (pers. comm.)
PAO947	*catA1 nar-9011 cnu-9011* FP2-	ROYLE, MATSUMOTO and HOLLOWAY (1981)
PAO948	*catA1 nar-9011 mtu-9002 tyu-9030* FP2-	ROYLE, MATSUMOTO and HOLLOWAY (1981)
PAO1039	*pur-67 cys-54 rif-96* FP2-	ROYLE, MATSUMOTO and HOLLOWAY (1981)
PAO1670	*pur-136 leu-8 rif-1* FP2–	CHANDLER and KRISHNAPILLAI (1974a)
PAO1790	*leu-9001 met-9011 pur-67 ilv-220* FP2-	This paper
PAO2001	*argH32 cml-2 str-39* FP2-	CHANDLER and KRISHNAPILLAI (1974a)
PAO2003	*argH32 cml-2 str-39 rec-2* FP2-	CHANDLER and KRISHNAPILLAI (1974a)
PAO2105	*met-9020 leu-9006 arg-9012 hex-9001* FP2-	H. MATSUMOTO (pers. comm.)
PAO2371	*met-9020 catA1 nar-9011 tyu-9026 cnu-9001* FP2-	H. MATSUMOTO (pers. comm.)
PAO2376	*met-9020 catA1 nar-9011 mtu-9002 tyu-9030* FP2-	H. MATSUMOTO (pers. comm.)

TABLE 1—Continued

Strain/plasmid/ phage	Genotype/phenotype	Source/reference
Plasmids		
FP2	Hg Pma Tra Cma IncP-8	JACOBY and SHAPIRO (1977)
R18	Cb Nm/Km Tc Phi(G101) Dps(PRR1, Pf3, PRD1, PR3, PR4) Tra IncP-1	CHANDLER and KRISHNAPILLAI (1974b) JACOBY and SHAPIRO (1977)
pMO850	Nm/Km Tc Phi(G101) Dps(PRR1, Pf3, PRD1, PR3, PR4) Tra Cma IncP-1	C. CROWTHER (pers. comm.)
R91-5	Cb Phi(G101) Dps(PRD1, PR3, PR4) Tra IncP-10	KRISHNAPILLAI (1979)
Sa	Cm Gm Km Sm Sp Su Tm Dps(PRD1, PR3, PR4) Tra Fi+ (RP1) IncW	JACOBY and SHAPIRO (1977)
R931	Sm Tc Hg Uv Phi(B3, B39, E79, F116L, G101, M6, PB1) Tra Fi+ (RP1) IncP-2	JACOBY and SHAPIRO (1977)
R38	Sm Su Tc Hg Pma Phi(B3, B39, D3, E79, G101, M6, PB1) Tra Fi+ (RP1) IncP-2	JACOBY and SHAPIRO (1977)
Phages	Characteristics	
E79*tv-2*	General transducing phage mutant of virulent phage E79	MORGAN (1979)
G101	General transducing phage; phage plating inhibited by R18 and R91-5	HOLLOWAY and VAN DE PUTTE (1968) CHANDLER and KRISHNAPILLAI (1977)
PRD1	Donor specific phage for IncP-1, R91-5 and some IncN and IncW plasmids	JACOBY and SHAPIRO (1977)

Host chromosome gene symbols are according to HOLLOWAY, KRISHNAPILLAI and MORGAN (1979), except for *mtu* = inability to use mannitol as sole carbon source and *bla* = resistance to carbenicillin due to beta-lactamase. The pairs of markers *met-9011*, *met-9020* and *tyu-9030*, *tyu-9026* are allelic by prototroph reduction tests (ROYLE, MATSUMOTO and HOLLOWAY 1981). Plasmid symbols are according to NOVICK *et al.* (1976) and JACOBY and SHAPIRO (1977), except for Cma = host chromosome mobilizing ability.

Transfer of plasmids: For frequency measurements, the quantitative method (FINGER and KRISHNAPILLAI 1980) was used, except that dilutions of the donor were used. For construction of plasmid-containing derivatives, the qualitative method (FINGER and KRISHNAPILLAI 1980) was used. FP2 acquisition by PAO429, and PAO431–PAO441 was detected by Hg^r transfer, R931, R38 or Sa by Sm^r transfer and R18 by Tc^r transfer. Donor contraselection was by CB, except for R18, which was by auxotrophy. R91–5 acquisition by the same strains was detected by premating donor and recipient on NA, re-isolation of the recipient by plating on MM supplemented with serine and CB and cross-streaking colonies against phage PRD1 (10^{10} pfu/ml). R91–5 derivatives were identified by sensitivity to this donor-specific phage (KRISHNAPILLAI 1979). This was necessary because cells harboring R91–5 confer only Cb^r, as do cells of PAO429 and PAO431–PAO441.

Tn1 transposition from the PAO chromosome to plasmids: R931, R38 or Sa was transferred into the strains having Tn*1* in the chromosome. Cb^r transfer was detected from these strains by mating with PAO2003 and plating 2×10^8 cells of donor and recipient on MM supplemented with arginine and CB. Plasmid transfer was separately measured by plating dilutions of the donor with 2×10^8 cells of PAO2003 on NA, incubating for 2.5 hr at 37°, washing off the cells and plating on MM supplemented with arginine and either TC (for R931 or R38 transfer) or CM (for Sa transfer). Premating on NA was necessary for expression of the resistance genes following transfer. The transposition frequency was expressed as the number of Cb^r transconjugants/Tc^r or Cm^r transconjugants, as described by BENNETT and RICHMOND (1976).

Plasmid DNA extraction, purification and agarose gel electrophoresis: The method used for plasmid extraction and purification from *P. aeruginosa* cells was essentially that of CLEWELL and HELINSKI (1969) and HUMPHREYS, WILLSHAW and ANDERSON (1975). Electrophoresis was through 0.7% agarose (Sigma) gels in a vertical slab gel apparatus. The gel dimensions were $18 \times 15 \times 0.35$ cm. The electrophoresis buffer was 40 mM Tris-HCl, 5 mM sodium acetate and 1 mM EDTA, adjusted with acetic acid to pH 7.6. The gels were run at 100 volts for 5 hr, stained in water containing 1 μg/ml ethidium bromide and photographed over a UV transilluminator (Ultraviolet Products, Inc.)

RESULTS

Evidence that the Cb^r transductants did not harbor R18: The transducing phage E79*tv-2* (MORGAN 1979) transduces R18 at a frequency of 10^{-6} Cb^r/pfu under optimal conditions (STOKES, MOORE and KRISHNAPILLAI 1981). During mutagenesis of R18, devised to select Tra^- mutants, 163 putative mutants were isolated at a frequency of about 1/700 of Cb^r transductants tested. The details are published elsewhere (STOKES, MOORE and KRISHNAPILLAI 1981). Of these, 151 were confirmed to be R18 mutants by a variety of criteria: resistance to kanamycin (Km^r) and tetracycline (Tc^r), entry exclusion (Eex^+), inhibition of the replication of phage G101 [Phi $(G101)^+$], amber suppressibility of their transfer deficiency, sensitivity to donor specific phages (Dps^+) or capability of complementing the transfer deficiency of other Tra^- mutants—all the phenotype properties characteristic of R18 (CHANDLER and KRISHNAPILLAI 1977; STOKES, MOORE and KRISHNAPILLAI 1981). However, 12 transductants (frequency of 1/9425 of the Cb^r transductants) were Km^s, Tc^s, Tra^-, Phi$(G101)^-$, Eex^-, Dps^- and did not possess extrachromosomal DNA, as judged by the failure to isolate supercoiled plasmid DNA. This was evidence that the Cb^r gene in these transductants was no longer associated with R18, and thus perhaps translocated to the bacterial chromosome. The twelve transductants were designated PAO429, PAO431–PAO441, and their Cb^r gene was designated *bla-429*, *bla-431–bla-441*,

respectively. In order to determine whether Cb^r in these transductants was chromosomally located, the Cb^r was tested for transducibility with phage G101 into RecA$^+$ (PAO2001) and RecA$^-$ (PAO2003) recipients. It was expected that, if the Cb^r gene in these transductants was in the chromosome, it should be transducible into the RecA$^+$ but not the RecA$^-$ recipient (CHANDLER and KRISHNAPILLAI 1974a). This was indeed found to be the case (data not shown). From these lines of evidence it was hypothesized that the Cb^r gene in each of the twelve transductants was located on the bacterial chromosome.

Chromosomal location for Cb^r: In order to confirm directly this hypothesis, the chromosomal locations of the Cb^r gene in these transductants was mapped genetically by segregation analysis of recombinants arising from conjugational crosses mediated by the plasmid FP2 (HOLLOWAY, KRISHNAPILLAI and MORGAN 1979). In view of the possibility that the Cb^r gene might have transposed into genes affecting prototrophy, it was first confirmed that PAO429, PAO431–PAO441 were still auxotrophic only for serine, the auxotrophy of the parent strain PAO2 into which the Cb^r was first transduced. For mapping, FP2 was transferred into all the transductional derivatives PAO429, PAO431–PAO441 to convert them to chromosomal donors. Each donor was crossed with a series of recipients having a range of markers distributed along the PAO chromosome (see Figure 3; HOLLOWAY, KRISHNAPILLAI and MORGAN 1979). In crosses selecting for prototrophic markers in the 0 to 40 min segment of the standard *P. aeruginosa* map (see Figure 3), segregation of Cb^r among the recombinant progeny was not observed from any of the strains PAO429, PAO431–PAO441 (data not shown). It was then decided to test markers distal to 40 min. Segregational analysis of recombinants from two- and three-factor crosses by conjugation showed that the Cb^r (*bla*) genes of all 12 transductants mapped in this region of the chromosome (Figures 1 and 2). The linkage distances with respect to single marker selections and co-inheritance of other markers are consistent with the location of the reference markers (HOLLOWAY, KRISHNAPILLAI and MORGAN 1979; ROYLE, MATSUMOTO and HOLLOWAY 1981). Secondly, with reference to the location of *bla*, it was found that recombinants simultaneously inheriting flanking markers nearly always inherited *bla*$^+$.

In order to determine whether these insertions were distinguishable by transductional linkage with markers adjacent to their location (Figures 1 and 2), phage G101 lysates were prepared on PAO429, PAO431–PAO441. These were used to transduce the appropriate recipients to *ami-200*$^+$, *hex-9001*$^+$, *catA1*$^+$, *mtu-9002*$^+$, *tyu-9030*$^+$, *cys-54*$^+$ and *cnu-9001*$^+$ and the transductants scored for *bla*$^+$ (200 transductants from each cross). It was found that the *bla*$^+$ of PAO432 and PAO435 were co-transducible with *catA1*$^+$ (57% and 51%, respectively), while all the other *bla*$^+$ insertions were not co-transducible with adjacent markers ($< 0.5\%$). The cotransducibility of two of the Tn*1* inserts with *catA1* further supports the conclusion that they are chromosomally located and that within clusters (*e.g.*, 440, 437, 432 and 435; see Figure 3) the locations are not identical

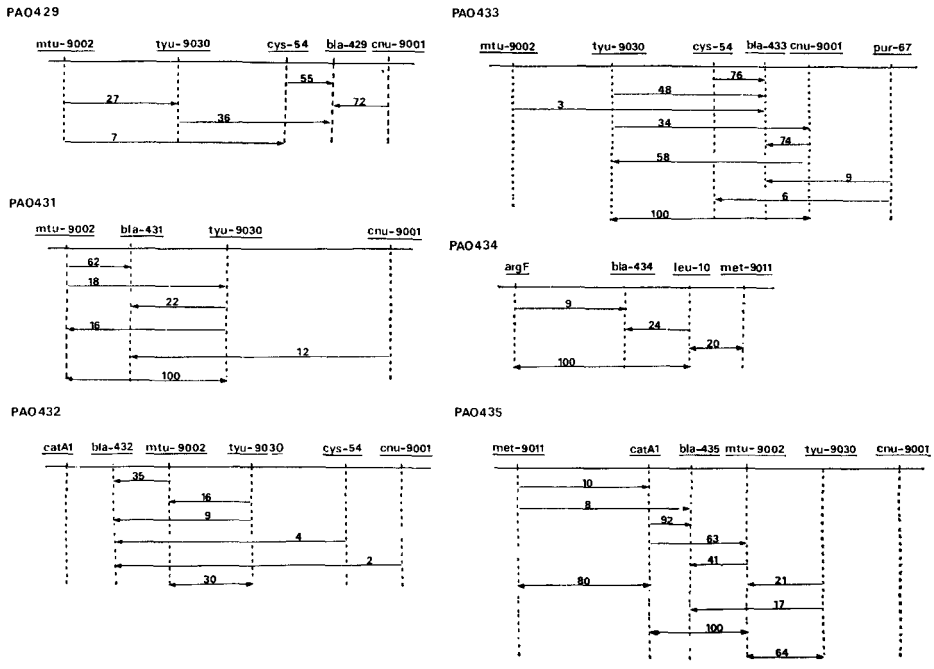

FIGURE 1.—Linkage data for the location of Tn*1* inserts in PAO429, POA431–PAO435. FP2+ donors of these strains, except PAO434, which was made pMO850+ (a Cb^s derivative of R68.45), were plate-mated with a series of recipients having markers in the region distal to 55 min of the *P. aeruginosa* PAO chromosome (see Figure 3). The recipients used were PAO25, PAO505, PAO947, PAO948, PAO1039, PAO2371 and PAO2376. Initially selected recombinants (100 of each class) were scored for co-inheritance of other markers including Cb^r (*bla*+). The unarrowed ends refer to the selected marker and the arrowhead to the unselected marker. The numbers refer to percentage co-inheritance. The double arrows refer to recombinants that simultaneously inherited both markers, and the number refers to the percentage of such double recombinants that were also *bla*+.

*Chromosome mobilization due to portable regions of homology created by Tn*1: An independent genetic confirmation of the Tn*1* insertions, as shown in Figures 1 and 2, was to determine whether the chromosomally inserted Tn*1* transposons could act as regions of homology for a plasmid having the same transposon (KLECKNER *et al.* 1979). If so, polarized chromosome transfer would ensue. Therefore, R18 was transferred into each of the strains PAO429, PAO431–PAO441, and chromosome-mobilizing ability (Cma) tested against recipients having the appropriate markers. For example, since the Tn*1* of PAO429 is located between *cys-54* and *cnu-9001* (Figure 1), the polarity of chromosome transfer from PAO429 (R18) would depend on the orientation of the transposon in the chromosome with that of the same transposon on R18 and the origin of transfer (*oriT*) of the plasmid. That is, in one orientation, *cys-54*+ would be transferred at much higher frequency than *cnu-9001*+; whereas, in the opposite orientation, the reverse result would be expected. This test would determine the

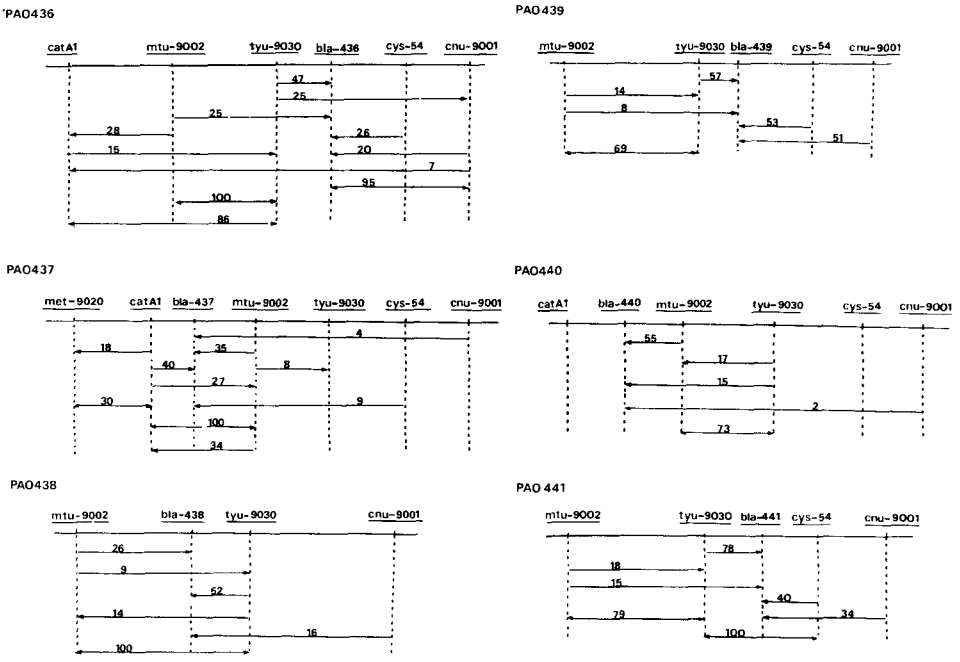

FIGURE 2.—Linkage data for the location of Tn*1* inserts in PAO436–PAO441. FP2+ donors of these strains were plate-mated with a series of recipients having markers in the region distal to 55 min of the *P. aeruginosa* PAO chromosome (see Figure 3). The recipients were PAO947, PAO948, PAO1039, PAO2371 and PAO2376. The rest of the details are as in the legend to Figure 1.

orientation of insertion of each of the Tn*1* transposons in the chromosomes of strains PAO429, POA431-PAO441 with respect to Tn*1* on R18.

The data (Table 2) support this proposition in every instance, and also confirm the genetic location of the insertions identified by segregational analysis in conjugational crosses. The orientations of these insertions (inferred from Table 2) are marked with arrows in Figure 3. Furthermore, the theory that for Cma to occur the Tn*1* has to be present in the donor chromosome was demonstrated by the absence of, or very low, Cma from PAO2, the parent strain of all the insertion derivatives (PAO429, PAO431–PAO441) carrying R18 against the markers tested (Table 2).

Another question concerned the possibility of determining the orientation of the Tn*1* transposon in another plasmid, namely R91–5 (KRISHNAPILLAI 1979), by the Cma test. R91–5 was transferred into PAO429, PAO434 and PAO436, and recombination for the appropriate markers was measured (Table 2). The results show that, in fact, Tn*1* in R91–5 is in the opposite orientation with respect to Tn*1* in R18, since the frequency of Cma for the pairs of markers flanking Tn*1* in PAO429, PAO434 and PAO436 is reversed. Therefore, the Tn*1* insertions in R18 and R91–5 are in opposite orientations with respect to *oriT* of the respective plasmids.

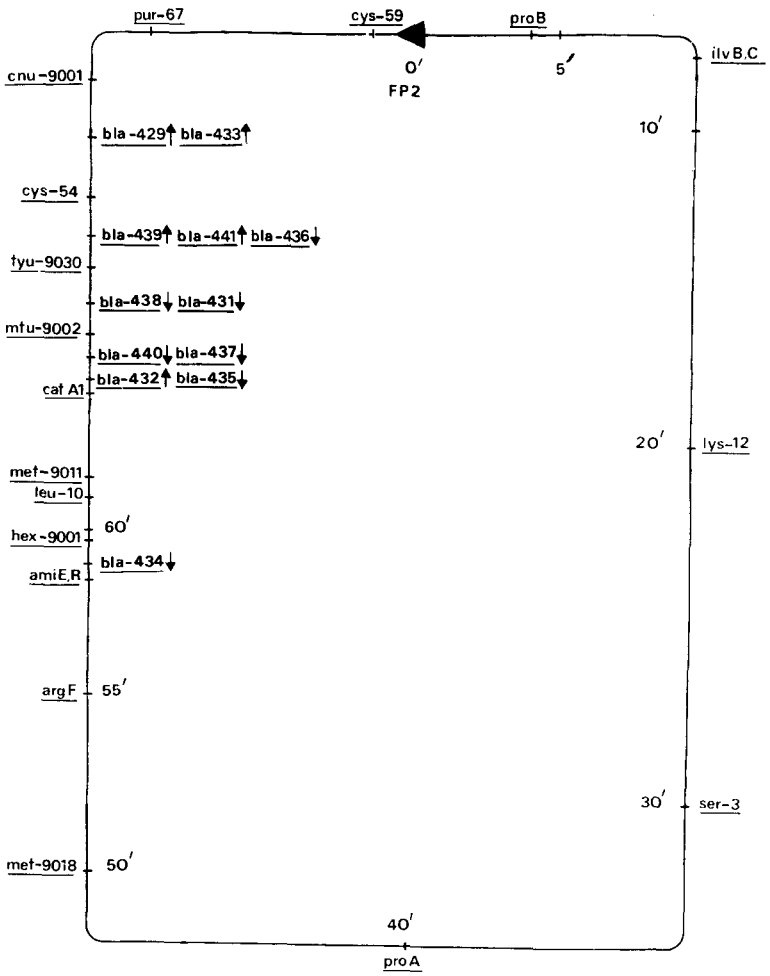

FIGURE 3.—Chromosomal location of Tn*1* insertions in the chromosome of *P. aeruginosa* strain PAO. The chromosome has recently been shown to be circular (ROYLE, MATSUMOTO and HOLLOWAY 1981). Only reference markers in the 0 to 55 min region are shown. A detailed map of PAO appears elsewhere (ROYLE, MATSUMOTO and HOLLOWAY 1981). The numbers inside the circle refer to marker distance (in min) established from interrupted-mating crosses with the plasmid FP2, and the arrow on the circle indicates the origin of transfer (HOLLOWAY, KRISHNAPILLAI and MORGAN 1979). The length of chromosome (in min) distal to 60 min has not yet been determined. The symbols *bla-429* to *bla-441* refer to the location of the Tn*1* insertions (*e.g.*, *bla-429* refers to the Tn*1* in strain PAO429), as determined from the data in Figures 1 and 2 and Table 2. The arrows adjacent to these numbers refer to the orientation of Tn*1* insertion as determined by Cma donor formation with the plasmid R18 due to the Tn*1* homology between the plasmid and chromosome (Table 2).

In addition, good recombination is detectable (4×10^{-5} recombinants/donor) at least as far as the *ilvB/C* locus, which is located at 8 min with respect to the origin of transfer by FP2 (Figure 3). Furthermore, good linkage was observed in this region, *e.g.*, in the cross PAO429(R91-5) × PAO1790, 10% of the *pur-*

TABLE 2

Host chromosome mobilization by plasmids R18 and R91-5

Donor	Recipient	Marker selected	Recombination frequency (per donor)
PAO429(R18)	PAO1039	*cys-54*+	2×10^{-6}
PAO429(R18)	PAO947	*cnu-9001*+	$< 1 \times 10^{-9}$
PAO431(R18)	PAO2376	*tyu-9030*+	1×10^{-7}
PAO431(R18)	PAO2376	*mtu-9002*+	$< 1 \times 10^{-9}$
PAO432(R18)	PAO2376	*catA1*+	2×10^{-7}
PAO432(R18)	PAO2376	*mtu-9002*+	$< 1 \times 10^{-9}$
PAO433(R18)	PAO1039	*cys-54*+	2×10^{-6}
PAO433(R18)	PAO947	*cnu-9001*+	$< 1 \times 10^{-9}$
PAO434(R18)	PAO2105	*hex-9001*+	5×10^{-5}
PAO434(R18)	PAO505	*ami-200*+	$< 1 \times 10^{-9}$
PAO435(R18)	PAO2376	*mtu-9002*+	7×10^{-8}
PAO435(R18)	PAO2376	*tyu-9030*+	6×10^{-7}
PAO435(R18)	PAO2376	*catA1*+	$< 1 \times 10^{-9}$
PAO436(R18)	PAO2376	*cys-54*+	4×10^{-5}
PAO436(R18)	PAO2376	*tyu-9030*+	$< 1 \times 10^{-9}$
PAO437(R18)	PAO2376	*mtu-9002*+	7×10^{-8}
PAO437(R18)	PAO2376	*tyu-9030*+	8×10^{-7}
PAO437(R18)	PAO2376	*catA1*+	$< 1 \times 10^{-9}$
PAO438(R18)	PAO2376	*tyu-9030*+	5×10^{-7}
PAO438(R18)	PAO2376	*mtu-9002*+	$< 1 \times 10^{-9}$
PAO439(R18)	PAO2376	*tyu-9030*+	1×10^{-6}
PAO439(R18)	PAO1039	*cys-54*+	$< 1 \times 10^{-9}$
PAO440(R18)	PAO2376	*mtu-9002*+	1×10^{-7}
PAO440(R18)	PAO2376	*tyu-9030*+	6×10^{-7}
PAO440(R18)	PAO2376	*catA1*+	$< 1 \times 10^{-9}$
PAO441(R18)	PAO2376	*tyu-9030*+	2×10^{-7}
PAO441(R18)	PAO1039	*cys-54*+	$< 1 \times 10^{-9}$
PAO429(R91-5)	PAO947	*cnu-9001*+	3×10^{-5}
PAO429(R91-5)	PAO1790	*pur-67*+	1×10^{-4}
PAO429(R91-5)	PAO1790	*ilv-220*+	4×10^{-5}
PAO429(R91-5)	PAO1039	*cys-54*+	6×10^{-7}
PAO434(R91-5)	PAO505	*ami-200*+	2×10^{-4}
PAO434(R91-5)	PAO2105	*hex-9001*+	1×10^{-7}
PAO436(R91-5)	PAO2376	*tyu-9030*+	6×10^{-4}
PAO436(R91-5)	PAO1039	*cys-54*+	2×10^{-7}
PAO2(R18)	PAO2105	*hex-9001*+	$< 1 \times 10^{-9}$
PAO2(R18)	PAO1039	*cys-54*+	8×10^{-9}
PAO2(R18)	PAO2376	*tyu-9030*+	2×10^{-9}
PAO2(R18)	PAO2376	*mtu-9002*+	$< 1 \times 10^{-9}$
PAO2(R18)	PAO2376	*catA1*+	4×10^{-9}
PAO2(R91-5)	PAO2105	*hex-9001*+	$< 1 \times 10^{-9}$
PAO2(R91-5)	PAO505	*ami-200*+	$< 1 \times 10^{-9}$
PAO2(R91-5)	PAO947	*cnu-9001*+	$< 1 \times 10^{-9}$
PAO2(R91-5)	PAO1039	*cys-54*+	2×10^{-7}
PAO2(R91-5)	PAO1790	*pur-67*+	3×10^{-7}
PAO2(R91-5)	PAO1790	*ilv-220*+	5×10^{-7}

Strains PAO2, PAO429, PAO431–PAO441 do not form recombinants ($< 10^{-9}$ recombinants/donor) in the absence of R18 or R91-5 in the donor.

67$^+$ recombinants were also *ilv-220*$^+$ (allelic with *ilvB/C*, see Figure 2; ROYLE, MATSUMOTO and HOLLOWAY 1981); whereas, 54% of *ilv-220*$^+$ recombinants were *pur-67*$^+$. The significance of this will be referred to in the DISCUSSION.

The orientations of the insertions (Figure 3) are based on data (Table 2) showing high and low recombination frequencies for the markers on either side of the insertions. That these frequencies reflect true recombinational differences, rather than efficiencies of marker recovery, is indicated by a comparison of homologous crosses using R18 or R91–5. For example, the frequency of *hex-9001*$^+$ and *ami-200*$^+$ was 5×10^{-5}/donor and $< 1 \times 10^{-9}$/donor, respectively, using PAO434 (R18) as the donor; whereas, it was 1×10^{-7} and 2×10^{-4}, respectively, when using PAO434(R91–5). That is, the high and low frequencies are reversed.

*Status of R18 or R91–5 in strains carrying chromosomal inserts of Tn*1: The Cma detected due to the Tn*1* homology between R18 and the chromosome (Table 2) might have been caused by integration of R18 into the bacterial chromosome; that is, by homologous recombination at the Tn*1* sequences, and the formation of stable Hfr donors. However, stable Hfr formation to explain Cma appeared to be unlikely because plasmid transfer (as measured by transfer of Cb^r) from R18 harboring derivatives of PAO429, PAO431–PAO441 to strain PAO1 was 10^{-1} to 10^{-2} Cb^r/donor. This frequency was not very different from the transfer frequency of 10^0 to 10^1 Cb^r/donor from PAO2 (R18) (the parent of the Tn*1* strains) to the same recipient. Similar results were also obtained when plasmid transfer was tested from PAO434 (R91–5) to PAO1. Although these data are consistent with the view that these donors have the plasmids integrating only transiently into the host chromosome, further experiments are needed to clarify this interpretation. However, they are formally analogous to Hfr donors of *Salmonella abony*, in which the plasmid F is infectiously transferable, while at the same time maintaining polarized chromosome transfer (MAKELA 1963).

*Retransposition of Tn*1 *from the chromosome to other replicons* (e.g., *plasmids*): Although the Tn*1* insertions in PAO429, PAO431–PAO441 have been shown to be chromosomally located (Figure 3), it is possible that one or more of the initial transpositions from R18 to the bacterial chromosome fail to retranspose from the chromosome to other replicons. This may be due to the generation of deletions affecting the terminal inverted repeats of Tn*1* (HEFFRON et al. 1977). To test this, and also to confirm physically that, when transposition occurs, it is accompanied by a discrete addition of DNA, *i.e.*, 3.2 megadaltons (md) HEFFRON *et al.* 1977), the IncW plasmid Sa (GORAI *et al.* 1979) was used as the recipient for Tn*1* transposition. Sa was transferred into PAO429, PAO431–PAO441, and the plasmid donors mated with PAO2003 by selecting Cb^r transfer. (PAO2003 was used because it is RecA$^-$.) Thus, mobilization of Tn*1* by Sa from the donor chromosome to the recipient chromosome, followed by homologous recombination mediated by the host *recA* gene, would be minimized. Transpositions to Sa would be preferentially identified since transposition of TnA is RecA-independent (KLECKNER, ROTH and BOTSTEIN 1977). Tn*1* transpositions from PAO429, PAO431, PAO434, PAO439, PAO440 and PAO441 were detected at

about 5×10^{-9} Cb^r transconjugants/R^+ transconjugant at 37°, but transpositions from PAO432, PAO433, PAO435, PAO436, PAO437 and PAO438 were not detectable ($< 10^{-9}$). The Sa::Tn*1* derivatives were confirmed to be Tra^+ Km^r Su^r Cm^r Cb^r (Gm^r Sm^r Sp^r and Tm^r, also coded by Sa, were not tested), which is the phenotype expected of Sa to which Tn*1* was transposed. Plasmid DNA from the Sa::Tn*1* derived from PAO429, PAO431, PAO434, PAO439, PAO440 and PAO441 was analyzed by agarose gel electrophoresis. The molecular weight standards used were plasmid DNA from R91–5 and Sa. R91–5 and Sa have molecular weights of 33.6 and 23 md, respectively (Moore and Krishnapillai, in preparation; Gorai *et al.* 1979). Such electrophoresis (Figure 4) showed that Sa::Tn*1* derived from PAO429, PAO431, PAO434, PAO439 and PAO441 has additions of DNA to the Sa molecule, *i.e.*, of the expected increase of 3.2 md, which is the molecular weight of the transposon (Heffron *et al.* 1977). However, it appears that

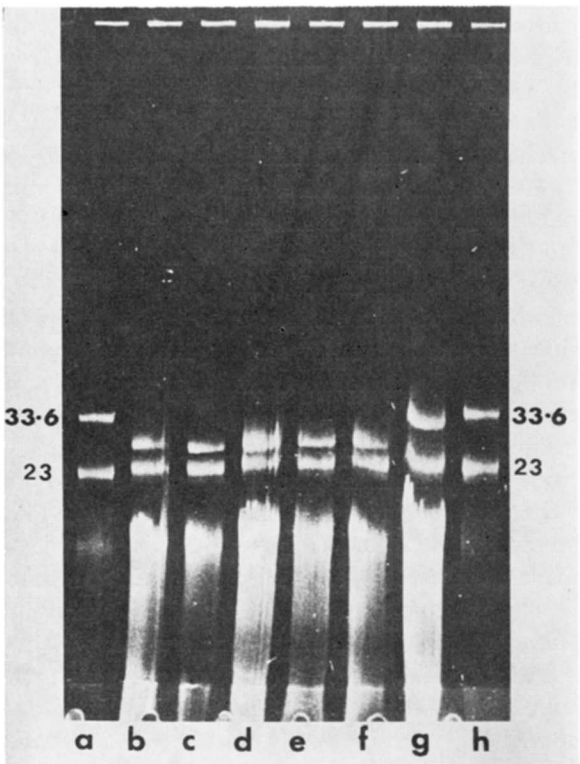

Figure 4.—Agarose gel electrophoresis of plasmid DNA. Lanes a and h are molecular weight standards, the upper being that of R91–5 DNA of 33.6 md and the lower of Sa DNA, 23 md. Lanes b, c, d, e, f and g are each mixtures of Sa DNA with DNA from Sa::Tn*1* constructed (as described in the text) from PAO441, PAO429, PAO431, PAO439, PAO434 and PAO440, respectively. In these, the lower band is that of Sa and the upper that of the corresponding Tn*1* derivative of Sa. In all cases except Sa::Tn*1* derived from PAO440, the molecular weight increase is consistent with a single copy addition of Tn*1* to Sa. With Sa::Tn*1* derived from PAO440, the molecular weight increase is consistent with a double-copy addition of Tn*1* to Sa, but other explanations, such as inversion or partial co-integration, have not been excluded.

the Sa::Tn*1* derivative from PAO440 is larger. This is consistent with either double transposition, inversion or partial co-integration, since its molecular weight is larger than the other Sa::Tn*1* derivatives, but smaller than that of R91–5, *i.e.*, 29.4 md *vs.* 33.6 md. The precise basis of the larger derivative has not yet been determined.

In view of the nondetectability of transposition from PAO432, PAO433, PAO435, PAO436, PAO437 and PAO438 to Sa, and also to determine whether transposition would occur with other plasmids, the IncP-2 plasmids R931 and R38 (JACOBY and SHAPIRO 1977) were tested as recipients for the Tn*1* transposition from strains PAO429, PAO431–PAO441. The protocol was similar to that used with Sa. It was found that transposition was detectable at 37° from each of the strains to both plasmids at a frequency of about 5×10^{-8} Cb^r transconjugants/R^+ transconjugant. The Tn*1* derivatives were all Tra^+ Hg^r Tc^r Cb^r (Sm^r coded by R931 and R38 was not tested). In order to confirm genetically that the Cb^r phenotype in the transconjugants was due to the transposition of Tn*1* to the IncP-2 plasmids, retransfer tests were conducted. One PAO2003 (R931::Tn*1*) and one PAO2003 (R38::Tn*1*) transconjugant derived from PAO429, PAO431–PAO441 was crossed with PAO1670 by selecting for Hg^r transfer. The resulting PAO1670 transconjugants were all Cb^r. One PAO1670 (R931::Tn*1*) and one PAO1670 (R38::Tn*1*) transconjugant derived from each of the previous crosses was recrossed with PAO1, this time selecting for Cb^r transfer. The resulting PAO1 transconjugants were all Hg^r. These results show that regardless of the selection used (Cb^r or Hg^r transfer), all transconjugants co-inherit Cb^r Hg^r. This confirms that the chromosomal Tn*1* transpositions from each of PAO429, PAO431–PAO441 are transposable to either R931 or R38 and, thus, that the initial transpositions of Tn*1* into the bacterial chromosome do not impair subsequent transposition to other replicons.

DISCUSSION

The most important conclusion to emerge from this investigation is that Tn*1* may be inserted into the bacterial chromosome of *P. aeruginosa* PAO. According to our knowledge, this is the first demonstration of such insertions in *P. aeruginosa*. Under the conditions used, insertion occurred at a frequency of 1/9425 of the Cb^r transductants tested. The method we have used to achieve this has not been reported before, and the twelve chromosomal transpositions were independent genetic events because of the transductional method used. This independence was also supported by genetic evidence of at least six different chromosomal locations. The donor of Tn*1*, the promiscuous plasmid R18, is physically identical to plasmid RK2 (BURKARDT, REISS and PUHLER 1979; STOKES, MOORE and KRISHNAPILLAI 1981). The latter has three distinct regions required for plasmid DNA replication and maintenance that encompass 4.1 kilobases (THOMAS, MEYER and HELINSKI 1980). Therefore, it is not surprising that mutagenesis of R18 by hydroxylamine could lead to the inability of this plasmid to survive as a replicon due to mutation in one of the essential genes. Thus, survival of Tn*1* was ensured by transposition into the bacterial chromosome by the method used,

namely the selection of Cb^r transductants. Although the method used in *P. aeruginosa* is novel, there are other methods available for chromosomal insertion in *E. coli* (RICHMOND and SYKES 1972; FOSTER, HOWE and RICHMOND 1975; DANILEVICH *et al.* 1978; ROBINSON *et al.* 1980).

On the basis of Cma donor formation, both orientations of Tn*1* insertion into the chromosome were found with about equal frequency. This situation in *P. aeruginosa* with respect to orientation is, in general, analogous to the behavior of Tn*10* when transposed from the phage P22 genome to the *S. typhimurium* chromosome (KLECKNER *et al.* 1979), or the behavior in *E. coli* of transposons Tn*3* and Tn*801* (which is identical to Tn*1* because of the heteroduplex identity of RP1 and RP4 according to BURKARDT, REISS and PUHLER 1979) when transposed from plasmid to plasmid (RUBENS, HEFFRON and FALKOW 1976; FOSTER *et al.* 1979). It is also similar to Tn*501* when transposed from plasmid to plasmid in *P. aeruginosa* or to Tn*802* (identical to Tn*801*) when transposed from the *E. coli* K12 chromosome to plasmid RP1, although there was a preponderance of one orientation when insertions occurred in particular regions of the recipient plasmid (GRINSTED *et al.* 1978). By contrast, Tn*7* always inserts in one orientation in *E. coli* (BARTH and GRINTER 1977). In general, the behavior of Tn*1* in *P. aeruginosa* appears to be quite similar to its behavior in *E. coli* or in *S. typhimurium* with respect to the parameters tested, *e.g.*, regional specificity (KRETCHMER and COHEN 1977; WEINSTOCK and BOTSTEIN 1979) and orientation of insertion (RUBENS, HEFFRON and FALKOW 1976; FOSTER *et al.* 1979; WEINSTOCK and BOTSTEIN 1979).

The dual orientation of Tn*1* insertions in the *P. aeruginosa* chromosome is particularly significant because these insertions occur in a region (distal to 55 min with respect to the plasmid FP2 origin of transfer (see Figure 3 and HOLLOWAY, KRISHNAPILLAI and MORGAN 1979), that has been refractory to detailed genetic mapping by interrupted mating crosses and linkage analysis. This is so because of (1) the paucity of markers in this region; (2) the single origin and direction of transfer by FP2 that is routinely used in recombination analysis; and (3) R68.45, the other plasmid extensively used in mapping, does not transfer more than 10 min of chromosome at a time, although it has multiple origins of transfer (HAAS and HOLLOWAY 1976). As shown here, both plasmids R18 and R91–5 could be exploited for mapping by taking advantage of Tn*1* homology between plasmid and chromosome. Furthermore, R91–5 transfers the chromosome across the site of the FP2 origin, linking markers in the distal and early regions (Figure 3). This, plus the fact that efficient conjugation takes place in liquid medium [unlike R68.45, which conjugates efficiently only on solid medium (HAAS and HOLLOWAY 1976)], has led to its use in interrupted mating experiments to map precisely the chromosome of PAO distal to the 55 min region (R. CROCKETT and A. MORGAN, personal communication).

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