Genet. Res., Camb. (1975), **25**, *pp.* 179–187
With 3 text-figures
Printed in Great Britain

Chromosomal location of prophage J51 in *Pseudomonas aeruginosa* strain PAO

By K. E. CAREY* and V. KRISHNAPILLAI

Department of Genetics, Monash University, Clayton Vic., 3168, Australia

(*Received* 3 *March* 1975)

SUMMARY

The UV-inducible prophage J51 has been mapped late on the chromosome of *P. aeruginosa* strain PAO, relative to the entry point of the sex factor FP2. This was determined following the analysis of the segregation of unselected markers in conjugational crosses between appropriately marked donor and recipient strains. A more precise location of about 50 min was obtained from the kinetics of increase in infectious centres due to zygotic induction during interrupted mating experiments.

1. INTRODUCTION

Chromosomal locations for prophages are known in a number of bacterial systems, e.g. *Escherichia coli* (Taylor & Trotter, 1972), *Salmonella typhimurium* (Sanderson, 1972) and *Bacillus subtilis* (Chow & Davidson, 1973). In most cases, single locations on the chromosome are indicated, but some phages have secondary sites that can be readily demonstrated, e.g. P2 (Sunshine & Kelly, 1968) and P27 (Bagdian & Makela, 1971); coliphage Mu-1 represents an extreme example of such multiple locations (Taylor, 1963; Bukhari & Zipser, 1972). The situation that occurs with coliphages λ, 434 and 82, where several distinguishable (though closely related phages) have prophage locations at the same region of the chromosome, appears to be less common (Taylor & Trotter, 1972).

Mapping of prophage sites in *Pseudomonas aeruginosa* strain PAO has not advanced to the detailed level found in the systems mentioned above, but already basic similarities are apparent. Prophage H90 shows linkage to chromosomal markers between 5 and 7 min on the strain PAO map (Pemberton & Holloway, 1972; Krishnapillai & Carey, 1972). In addition, zygotic induction of H90 has been demonstrated, and the consequent genetic effect of reduced recombinant yield for distal markers utilized for precise mapping (Carey & Krishnapillai, 1974). The present report deals with mapping of the prophage site of a second phage, J51, late on the strain PAO chromosome.

* Department of Microbiology, Medical School, University of Michigan, Ann Arbor, Michigan 48104, U.S.A.

2. MATERIALS AND METHODS

(i) *Media.* These have been described previously (Krishnapillai, 1971).

(ii) *Bacterial strains.* See Table 1.

(iii) *Bacteriophage.* J51 was isolated from a wild-type strain of *P. aeruginosa* (obtained from a clinical source in a Melbourne hospital) as described by Krishnapillai (1971). Phage and bacterial handling techniques were essentially those described by Adams (1959).

(iv) *Interrupted mating technique.* The method using nalidixic acid for interruption has been described (Carey & Krishnapillai, 1974).

(v) *Plate mating conjugation technique.* This has been described (Stanisich & Holloway, 1969).

Table 1. *Bacterial strains*

Strain	Genotype	Reference/derivation
PAO1	Prototroph, *ch12* FP2−	Krishnapillai (1971)
PAO873	*thi1 his151 pyr21 thr1 pur66 ese14* FP2−	Pemberton & Holloway (1972)
PAO1751	*trp1 arg2 aerR2 aerS str* $F116^R$ $G101^R$ FP2−	M78*
PAO2516	*thi1 his151 pyr21 thr1 pur66 ese14 na13* FP2−	PAO873 made nalidixic acid resistant
PAO2517	*trp1 arg2 aerR2 aerS str* $F116^R$ $G101^R$ *na14* FP2−	PAO1751 made nalidixic acid resistant
PAO2528	Prototroph, *ch12* (J51) $^+$FP2−	PAO1 made lysogenic for phage J51
PAO2531	*met28 ilv202 str2* (J51) $^+$FP2+	PAO2601 made lysogenic for phage J51
PAO2601	*met28 ilv202 str2* FP2+	V. Stanisich (personal communication)

* The genotype of this strain is *trp1 arg2 aerR2 str* $F116^R$ FP2− (Kageyama, 1970*a*) and was obtained from Dr M. Kageyama. We have found this strain to be also resistant to the transducing phage G101. The strain was mutated with nitrosoguanidine to obtain a mutant defective in aeruginocin S (*aerS*) synthesis and the derivative was designated PAO1751.

Abbreviations: *arg*, *his*, *ilv*, *met*, *pur*, *pyr*, *thi*, *thr*, *trp* refer to nutritional requirements for arginine, histidine, isoleucine + valine, methionine, purine, pyrimidine, thiamine, threonine, tryptophan, respectively. *aerR2* and *aerS* refer to inability to produce active aeruginocin of the R and S type, respectively. *ch1*, *na1*, *str* refer to resistance to chloramphenicol, nalidixic acid and streptomycin, respectively. *ese*, $F116^R$ and $G101^R$ refer to resistance to phages E79, F116 and G101 respectively. FP2 is the *P. aeruginosa* sex factor.

3. RESULTS

(i) *Characteristics of phage J51*

After 16 h incubation at 37 °C, phage J51 forms solid-centred plaques (typical of temperate phages) about 1 mm in diameter in soft (0·6 %) agar seeded with strain PAO1. Prophage J51 is inducible by UV irradiation, yielding a 10^3-fold increase in plaque-forming units in the extracellular medium after 300 erg/mm^2 treatment of a J51 lysogen. This phage represents a new serogroup (J) for this laboratory, being serologically unrelated to phages previously characterized

(Holloway, Krishnapillai & Stanisich, 1971; Carey & Krishnapillai, 1974), both on the basis of insensitivity of J51 to sera specific for phages of other groups, and also by failure of J51-specific antiserum to neutralize such phages.

(ii) *Mapping of prophage J51 by unselected marker co-inheritance in plate mating conjugations*

(1) *Recipient: PAO873* thi1, his151, pyr21, thr1, pur66, ese14, *FP2⁻*. *Donor: PAO2601* met28, ilv202, str2, *FP2⁺*.

(*a*) *Non-lysogenic donor × non-lysogenic recipient* (*Table 2*(*a*)). This recipient strain possesses markers which are distributed throughout the mapped region of the strain PAO chromosome (Pemberton & Holloway, 1972; Fig. 1). The markers *thr1* and *pur66* have been reported to be co-transducible and are located at about 44 min. Selection for donor markers and measurement of co-inheritance between markers in this cross formed the background comparison for mapping prophage J51 in this strain.

Table 2. *Unselected marker co-inheritance values* (%) *for prophage J51 and auxotrophic markers in plate mating crosses*

	Selected donor allele	Unselected donor allele co-inheritance (%)						No. tested
			Thi⁺	His⁺	Pyr⁺	Thr⁺	Pur⁺	
(*a*)	Thi⁺		*	5	1	0	0	96
	His⁺		37	*	1	0	0	96
	Pyr⁺		25	27	*	0	0	96
	Thr⁺		6	1	6	*	54	96
	Pur⁺		11	3	4	58	*	96
		(J51)⁺	Thi⁺	His⁺	Pyr⁺	Thr⁺	Pur⁺	
(*b*)	Thi⁺	0	*	6	0	0	0	96
	His⁺	0	42	*	0	0	0	96
	Pyr⁺	1	17	21	*	0	0	96
	Thr⁺	13	4	1	5	*	52	96
	Pur⁺	15	10	3	3	63	*	96

Recipient: PAO873 *thi1*, *his151*, *pyr21*, *thr1*, *pur66*, *ese14*, FP2⁻.
Donor: PAO2601 *met28*, *ilv202*, *str2*, FP2⁺.
(*a*) Non-lysogenic donor × non-lysogenic recipient.
(*b*) (J51) lysogenic donor × non-lysogenic recipient.

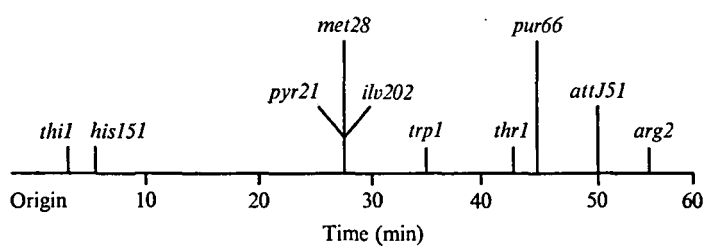

Fig. 1. Locations of genetic markers used in these studies, based upon the strain PAO chromosome map of Pemberton & Holloway (1972).

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(*b*) *Lysogenic donor* × *non-lysogenic recipient* (*Table 2*(*b*)). A location of prophage J51 that is distal to *pur66* is suggested by these data, as co-inheritance with this marker is greater than with any of the other markers, and is much less than the co-inheritance between *thr1* and *pur66*. This then suggests a map order of origin – *thil* – *his151* – *pyr21* – *thr1* – *pur66* – prophage J51.

(2) *Recipient: PAO1751* trp1, arg2, aerR2, aerS, str, *F116*^{R}, *G101*^{R}, *FP2*$^{-}$. *Donor: PAO2601.*

Table 3. *Unselected marker co-inheritance values* (%) *for prophage J51 and auxotrophic markers in plate mating crosses*

	Selected donor allele	Unselected donor allele co-inheritance (%) (J51)$^{+}$	Trp^{+}	Arg^{+}	No. tested
(*a*)	Trp^{+}	30	*	34	96
	Arg^{+}	82	40	*	96
		(J51)$^{-}$	Trp^{+}	Arg^{+}	
(*b*)	Trp^{+}	35	*	41	96
	Arg^{+}	71	43	*	96

Recipient: PAO 1751 *trp1, arg2, aerR2, aerS, str*, F116^{R}, G101^{R} FP2 –.
Donor: PAO 2601 *met28, ilv202, str2*, FP2 +.
(*a*) Lysogenic donor × non-lysogenic recipient.
(*b*) Non-lysogenic donor × lysogenic recipient.

(*a*) *Lysogenic donor* × *non-lysogenic recipient* (*Table 3*(*a*)). Co-inheritance values of prophage J51 with each of the two markers in this cross are quite different. The higher linkage to Arg + (82 %) than to Trp + (30 %) suggests a location closer to *arg2*. *trp1* has been mapped at about 33 min (Kageyama, 1970*a*, *b*; Pemberton & Holloway, 1972), but *arg2* appears to be too late on the chromosome to be accurately mapped by time of entry in interrupted mating experiments. This *arg* locus appears to correspond to the *argF* locus on the basis of growth response to the intermediates in arginine biosynthesis (D. Haas, personal communication). *argF* has been genetically mapped in this region of the chromosome (Brake & Holloway, personal communication). However, it has not been possible to test the possible genetic identity of these two loci by transductional tests because both the transducing phages F116L and G101 fail to plate on PAO1751 (presumably due to non-adsorption).

(*b*) *Non-lysogenic donor* × *lysogenic recipient* (*Table 3*(*b*)). The values presented for this reciprocal cross involving transfer of 'non-lysogeny' are similar to those presented above. The previous conclusion of a late site for prophage J51 is supported by these data from the reciprocal cross.

Plate mating experiments in *P. aeruginosa* strain PAO present various intrinsic problems in the study of late regions of the chromosome. The low level of recombinant recovery and co-inheritance of markers, associated with fluctuations in values, prevent accurate mapping. In addition, wide variations in recombinant recovery and co-inheritance of markers in different strains prevents close inter-strain comparisons (compare co-inheritance values in Table 2 and 3 for markers of

comparable distance from the FP2 origin in strains PAO873 and PAO1751 respectively). For these reasons it is not possible to estimate the location of prophage J51 from these plate mating data, other than to propose a position distal to *thr1* and *pur66* and probably proximal to *arg2*.

Direct detection of release of induced phage particles during interrupted matings should present a sensitive method of mapping the time of entry of the prophage of the transferred chromosomal segment. To maximize the chances of detecting infectious particles, a method was devised which allowed removal of background phage particles to as low as 3×10^2 pfu/ml, with no detrimental effects on recombinant formation. In essence, the method involves interruption with nalidixic acid (400 μg/ml) and filtration of the mixture through 0·45 μm Millipore membrane filters which retain cells but not phage particles. Tests showed that the donor

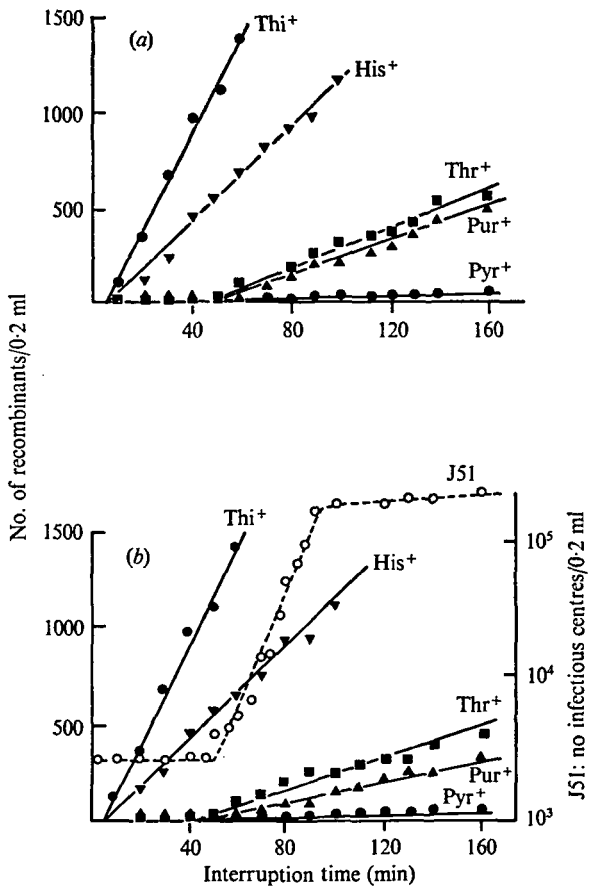

Fig. 2. Interrupted mating experiments to map prophage J51 using nalidixic acid for interruption. Background phage levels were reduced following interruption by filtration. Selection for nutritional markers was on supplemented minimal media. Infectious centre increase was detected by plating in soft nutrient agar overlays, seeded with indicator bacteria. Recipient: PAO 2516 *thi1*, *his151*, *pyr21*, *thr1*, *pur66*, *ese14*, *nal3*, FP2−. Donor: PAO 2601 *met28*, *ilv202*, *str2*, FP2+. (*a*) Non-lysogenic donor × non-lysogenic recipient. (*b*) (J51) lysogenic donor × non-lysogenic recipient.

lysogenic for J51 was not induced by nalidixic acid at the levels used in these experiments (see the work of Cowlishaw & Ginoza (1970) on induction of λ lysogens).

Recipient strains derived from PAO873 and PAO1751 were resistant to 500 μg/ml of nalidixic acid (PAO2516 and PAO2517 respectively). Donor strains were all sensitive to the contra-selective agent.

Fig. 2(*a*) and (*b*) show the time of entry curves for donor alleles with strain PAO2516 as recipient. The control cross with the non-lysogenic donor is presented in Fig. 2(*a*), while (*b*) utilizes a donor lysogenic for J51. As was expected, recovery of recombinants for late markers is very low. The recovery of Pyr$^+$ is also depressed considering its mapped position at about 29 min, but this is due to the closeness of

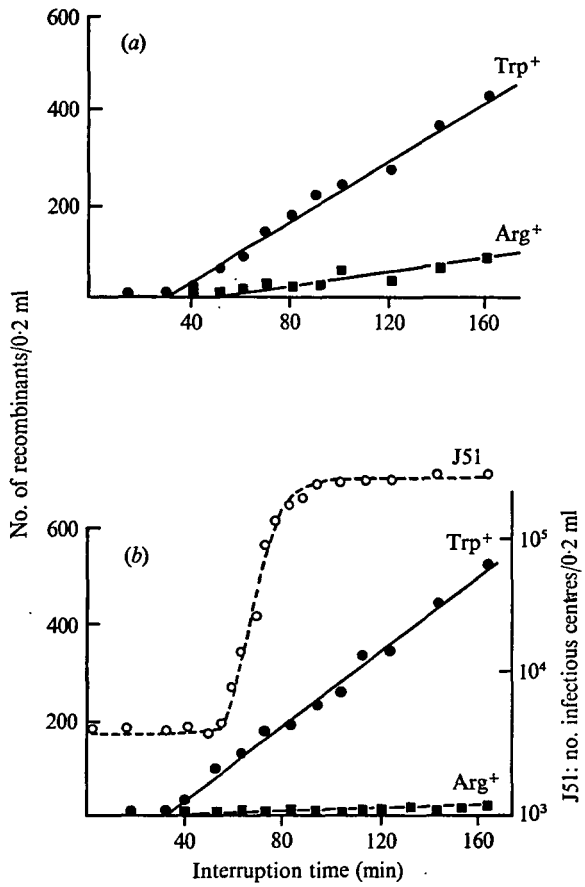

Fig. 3. Interrupted mating experiments, to map prophage J51 using nalidixic acid for interruption. Background phage levels were reduced following interruption by filtration. Selection for nutritional markers was on supplemented minimal media. Infectious centre increase was detected by plating in soft nutrient agar overlays, seeded with indicator bacteria. Recipient: PAO 2517 *trp1*, *arg2*, *aerR2*, *aerS*, *str*, F116^R, G101^R, *na14*, FP2−. Donor: PAO 2601 *met28*, *ilv202*, *str2*, FP2+. (*a*) Non-lysogenic donor × non-lysogenic recipient. (*b*) (J51) lysogenic donor × non-lysogenic recipient.

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pyr21 and the donor contraselective markers *met28* and *ilv202* (29 min) which necessitates crossover events within the restricted region between these genes to allow viable recombinants. Alternative donors with markers in other regions show higher levels of Pyr$^+$ recovery (Carey, 1974).

What is important, however, is that the yield of infectious centres increases rapidly over the following 40 min to reach a level that is about 100 times the basal level (Fig. 2(*b*)). Very little increase is observed after that plateau level is reached.

The apparent zygotic induction of J51 was further investigated using strain PAO2517 as recipient. Fig. 3(*a*) is the non-lysogenic donor control cross, (*b*) shows the cross using the donor lysogenic for J51. Again, the cross with the lysogenic J51 donor shows an increase in infectious centres at about 50 min. From these data, it would seem that Arg$^+$ enters at about 55 min, although this estimate is not a precise one, as is discussed below.

The low level of recombinant recovery for late markers in these crosses, precludes precise analysis of proximal/distal relationships with prophage J51 on the basis of decreased recombinant recovery. This method was successfully employed in the case of prophage H90 which was mapped between 5 and 7 min in which region recombinant recovery was high and was noticeably depressed for distal markers (Carey & Krishnapillai, 1974). However the slight reduction in the recovery of Arg$^+$ when the donor is lysogenic (Fig. 3(*b*)) in comparison to when it is non-lysogenic (Fig. 3(*a*), presumably because of zygotic induction) is consistent with a distal location for *arg2* with respect to *attJ51*.

Pemberton (1971) has argued for an error of ± 2 min in estimating the times of entry of markers using the technique of interrupted mating in *P. aeruginosa*. It can be seen that with increasing lateness of the transferred region, this error increases markedly; the time of entry of *arg2* appears to be later than prophage J51 at about 55 min. This figure, however, is the average of ten experiments with a range of estimates from 50 to 62 min. Similarly, the value of 50 min for prophage J51 is the average of a range from 48 to 53 min. The smaller range probably reflects the greater sensitivity of mapping by infectious centres.

4. DISCUSSION

The prophage of the UV-inducible phage J51 has been found to be linked to the bacterial chromosome. Evidence from unselected marker co-inheritance analyses in plate mating crosses had indicated that prophage J51 is located very late on the strain PAO chromosome. This has been strongly supported by the detection of increasing infectious centres during zygotic induction in interrupted mating experiments which has allowed a more precise estimation, placing prophage J51 at about 50 min. At the present time it is not known why some indication of the zygotic induction of prophage J51 did not appear in the plate mating conjugation experiments, e.g. low linkage of J51 to the nearby auxotrophic markers would have been expected when a lysogenic donor is used (Table 3(*a*)), by comparison with the reciprocal combination (Table 3(*b*)). Similarly, it might be expected that

some difference in linkage values should have been obtained for nearby markers in Table 2, comparing the case of a non-lysogenic donor with that of a lysogenic donor ((*a*) and (*b*) respectively). Since it would appear that the zygotic induction system of prophage J51 is relatively efficient, yielding a 100 fold increase in infectious centres, it can only be assumed at this point, that conditions for zygotic induction are far from optimal in plate mating crosses as opposed to the interrupted mating crosses. It is possible that multiple rounds of mating which may occur readily during plate matings, mask the zygotic induction events, utilizing recipients that have survived a potential zygotic induction situation following introduction of the prophage.

It has become apparent that prophage location in *P. aeruginosa* is similar to that in other bacterial systems, in that chromosomal sites are a common occurrence. Krishnapillai & Carey (1972) presented linkage data that indicated that the prophage of H90 was located between 7 and 13 min on the strain PAO map. Prophage H90 was more precisely mapped between 5 and 7 min by exploiting the effects of zygotic induction of this prophage (Carey & Krishnapillai, 1974). Further studies have shown that a group of 12 other prophages which appeared to be indistinguishable from H90 on numerous criteria, are similarly located between 5 and 7 min (Carey, 1974). Interestingly, this group of phages appear to be partially defective in assembly as evidenced initially by an inability to obtain high titre phage preparations (greater than about 10^8 pfu/ml) by a variety of methods, and then by electron microscopic observations of H90 lysates which showed that only approximately 1 % of component parts are assembled into intact phage particles (Carey, 1974).

The present paper is further support for the contention that chromosomal prophage locations in *P. aeruginosa* are not likely to be rare. Another phage, J84, which appears to be very closely related to J51, with the exception that while it is UV inducible it does not show zygotic induction, has been found to yield almost identical co-inheritance values in plate mating conjugations, to those found for J51 (Carey, 1974). (While failure to exhibit zygotic induction on the part of prophage J84 might suggest an extrachromosomal location for this prophage, the consistent co-inheritance with chromosomal markers late on the map and not with early markers, would argue for a chromosomal site in that region).

In the context of chromosomal sites for prophages, it is probably germain to mention that the 'defective phage tail' R-type aeruginocin has been mapped at about 33 min on the strain PAO map (Kageyama 1970*a*, *b*; Pemberton & Holloway, 1972). Accompanying morphological studies (e.g. Ito, Kageyama & Egami, 1970) led to the suggestion that this aeruginocin particle is in fact a defective phage, and that *aerR2* is the chromosomal site of this defective phage.

Although the occurrence of chromosomal prophage locations and of zygotic induction phenomena in *P. aeruginosa* suggest close parallels with other phage-bacterial interactions, the crucial central issue of whether prophage insertion in *P. aeruginosa* takes place via the Campbell model (1963), is only a working theory at the present time.

This work was supported by the Australian Research Grants Committee. We appreciate Professor B. W. Holloway's and Dr R. Olsen's comments on the manuscript. K. E. Carey is the holder of a Commonwealth Postgraduate Research Award.

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