JOURNAL OF BACTERIOLOGY, Feb. 1986, p. 448–452
0021-9193/86/020448-05$02.00/0

Vol. 165, No. 2

Specialized Transduction of *Pseudomonas aeruginosa* PAO by Bacteriophage D3

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Received 26 July 1985/Accepted 18 November 1985

D3, a temperate bacteriophage of *Pseudomonas aeruginosa* PAO, was found to specifically transduce the alleles *met-49* and *met-117*. Induction of established lysogens with UV light was necessary for the production of transducing lysates. Transduced cells were immune to superinfection by phage D3 and could give rise to high-frequency transducing lysates. Cotransduction of these two alleles could not be demonstrated. *met-117* was mapped to 26 min on the PAO genetic map. Complementation studies using the generalized transducing phage Fll6L indicated that *met-49* is an allele of *met-9011* which maps at 55 min. The integrated D3 prophage was shown to be coinherited with *met-117* and with *met*-49.

Although there are many temperate phages of *Pseudomonas aeruginosa* and the frequency of lysogeny among *P. aeruginosa* strains is probably close to 100%, no cases of prophage-linked specialized transduction in this species have been reported (9, 10, 13). D3, first isolated in 1960 by Holloway et al. (11), is a temperate bacteriophage of *P. aeruginosa* PAO which has a polyhedral head and a prominent tail with six knoblike projections (20). It contains a linear DNA molecule of molecular weight 4.4×10^7 (20). The D3 prophage can be induced to lytic growth with UV light (14). Genetic recombination has been demonstrated in mixed infections of plaque morphology mutants of D3 (3). The data presented here suggest that D3 acts as a specialized transducing phage of *P. aeruginosa* and that the D3 prophage integrates into the *P. aeruginosa* PAO chromosome at at least two sites.

MATERIALS AND METHODS

Bacteria and bacteriophages. The strains used in this study are described in Table 1. All are derivatives of *P. aeruginosa* PAO. Bacteriophages F116 and D3 were obtained from B. W. Holloway. F116L was obtained from J. A. Shapiro.

Media. Luria broth was purchased from GIBCO Laboratories, Madison, Wis. *Pseudomonas* minimal medium (PMM [18]) was supplemented with 0.2% glucose and amino acids as appropriate at 50 μg/ml. Ornston-Stanier medium was as described (21), except nitroloacetic acid was omitted. To make plates of any of these media, agar was added to 1.5%.

Preparation of transducing lysates. F116 and F116L phages were prepared by the plate lysate method of Miller and Ku (18). For use in transduction experiments, F116 or F116L was passed twice consecutively through the donor strain. D3 was obtained by induction of the prophages from lysogenic *P. aeruginosa* strains by exposure to 3.5 J/m^2 of UV light.

Conjugations. Time-of-entry experiments were performed according to the method of Haas and Holloway (6). They were carried out on minimal medium plates, supplemented as needed with amino acids. PMM was used when selecting for amino acid markers, and Ornston-Stanier medium was used for carbon source markers. Matings were interrupted

TABLE 1. Bacterial strains

Strain	Genotype[a]	Source or reference
PAO1	Prototroph	9
PAO25	*argF leu-10*	22
PAO283	*his-1 lys-12 met-28 trpC ese-1*	18
PAO303	*argB21*	18
PAO381	*leu-38 str-2*; FP2	22
PAO515	*met-9011 amiE200 nalA5*	D. Haas
PAO881	*arg-164 leu-8 pur-136 ese-21*	22
PAO1000	*trpA,B*::pME319 *his-301 str-1*	6
PAO1670	*leu-8 pur-136 rif-1 ese-21*; pMO171	B. W. Holloway
PAO2375	*met-9020 catA1 mtu-9002 nar-9011*	B. W. Holloway
PTO6015	*leu-10 rec-102 strA*; pME134[b]	D. Haas
RM17	*leu-38 str-2* [D3]; FP2	D3 lysogen of PAO381
RM34	*cys-72 met-28*	D. H. Calhoun
RM40	*lys-12 met-28 trpC6 pur-600 str-910*	18
RM42	*trpB*	D. H. Calhoun
RM44	*trpA*	1
RM45	*met-49*	1
RM46	*met-117*	1
RM48	*met-16*	1
RM49	*met-417*	1
RM51	*cys-411*	1
RM172	*leu-38* [D3]; FP2	RM17 × RM512
RM230	*argF leu-10 nal-905*	Nal^r derivative of PAO25
RM268	*met-9020 catA1 mtu-9002 nar-9011 nal-906*	Nal^r derivative of PAO2375
RM406	*lys-12 met-28 trpC6 pur-600 nal-901 str-910*	Nal^r derivative of RM40
RM512	Prototroph [D3]	D3 lysogen of PAO1
RM2030	*ser-3*; FP110	B. W. Holloway
RM2062	*met-49 nal-903 dtr-901*	D3^r derivative of RM45
RM2063	*met-117 nal-904 dtr-902*	D3^r derivative of RM46

[a] Nomenclature according to Royle et al. (24), with the addition of *dtr*, which indicates D3 resistance and [D3] which indicates that the strain is lysogenic for prophage D3. Nal^r indicates resistance to nalidixic acid, and D3^r indicates resistance to infection by phage D3.

[b] Integrated into the chromosome at 72 min.

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TABLE 2. D3-mediated transduction of various alleles

Recipient strain	Marker tested	Map position[a] (min)	MOI[b]	Transductants/ PFU
PAO303	*argB21*	21	2.6	$<10^{-9}$
PAO881	*pur-136*	25	2.4	$<10^{-9}$
RM46	*met-117*	26	3.3	8.7×10^{-7}
RM42	*trpB*	27	1.4	$<10^{-9}$
RM44	*trpA*	27	0.4	$<10^{-9}$
PAO283	*met-28*	28	5.0	$<10^{-9}$
PAO1670	*leu-8*	42	1.2	$<10^{-9}$
RM45	*met-49*	55	4.0	3.6×10^{-7}
PAO283	*his-1*	95	0.75	$<10^{-9}$
RM34	*cys-72*	?	1.0	$<10^{-9}$
RM48	*met-16*	?	2.0	$<10^{-9}$
RM49	*met-417*	?	5.3	$<10^{-9}$
RM51	*cys-411*	?	3.6	$<10^{-9}$

[a] Map positions are from Royle et al. (24) except *met-49* and *met-117*, which were determined in this study.
[b] MOI, multiplicity of infection.

by spreading the plates with nalidixic acid to a final concentration of 250 μg/ml.

For coinheritance studies, uninterrupted matings were carried out as described by Miller and Ku (18). Transconjugants inheriting donor prototrophic markers were selected on appropriately supplemented minimal media and purified. Purified transconjugants were scored for coinheritance of the lysogeny/nonlysogeny phenotype by testing for spontaneous release of phage on an indicator strain (PAO1).

Transductions. Cells to be transduced were grown in 10 ml of Luria broth to early log phase. They were spun down and resuspended in 5 ml of TNM buffer (0.01 M Tris, 0.15 M NaCl, 0.01 M $MgSO_4$; pH 7.4). Cells were then mixed with phage and incubated at 37°C for 10 min. The transduction mixtures were plated in duplicate on selective media at desired dilutions. Transductants appeared after incubation for 2 days at 37°C.

RESULTS

Transductions of specific methionine alleles by phage D3. We tested a number of genetic loci to determine whether they are transduced by bacteriophage D3 (Table 2). Each of the strains tested was sensitive to phage D3, and each of the alleles tested was transducible by the generalized transducing phage F116 (11, 18) at an equal efficiency (data not shown). Only two methionine alleles, *met-49* and *met-117*, were found to be transduced by D3. While the frequency of transduction was found to vary with the multiplicity of

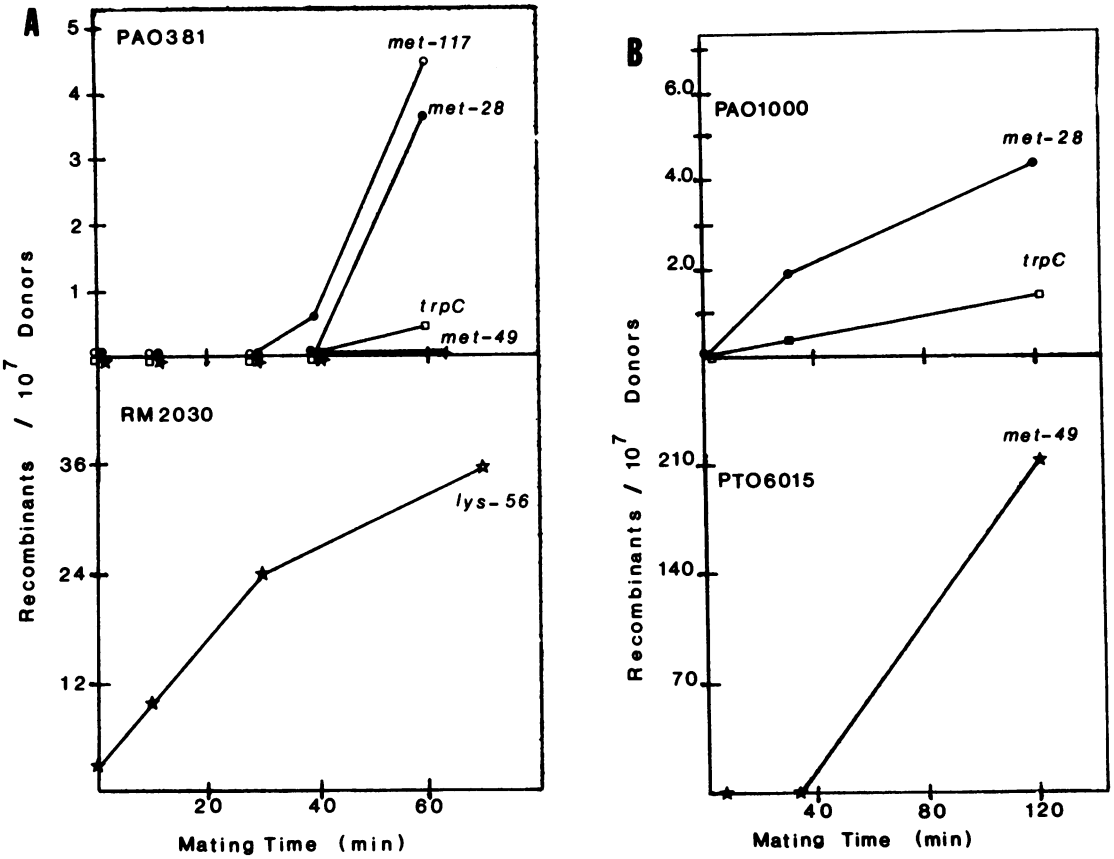

FIG. 1. Interrupted matings. Plate matings were interrupted with nalidixic acid after various time intervals. Results for each selected allele are presented as the frequency of recombinants observed as a function of mating time. The strains used are listed below, followed by the alleles selected. No recombinants were recovered for alleles that are listed as having been selected but that do not appear on the corresponding graph. (A) Mapping *met-117*: PAO381, an FP2 donor, was crossed with RM2062 (*met-49*), RM2063 (*met-117*), and RM406 (*met-28 trpC*). RM2030, an FP110 donor, was crossed with RM2062 (*met-49*), RM2063 (*met-117*), and RM406 (*met-28 lys-56*). (B) Mapping *met-49*: PAO1000 and PTO6015 were crossed with RM2062 (*met-49*), RM2063 (*met-117*), RM230 (*argF*), and RM406 (*met-28 trpC lys-56*).

TABLE 3. Transductional frequencies of *met-49* and *met-117*

Marker	Experiment no.	Lysate no.[a]	MOI[b]	No. of transductants[c]	Transductants/ 10^7 PFU
met-49	1	1	3.9	531	3.6
	2	1	13.8	31	0.2
	3	2	5.0	2,120	6.2
	4	3	1.5	192	6.4
	5	3	3.3	132	1.3
met-117	1	1	3.2	874	8.7
	2	1	10.7	1,600	11
	3	2	1.7	4,000	12
	4	3	2.1	5,280	180
	5	3	32.3	120	0.2

[a] D3 was induced by UV irradiation from RM512.
[b] MOI, multiplicity of infection.
[c] Number of prototrophic colonies above the level of spontaneous revertants.

infection and with the phage lysate used (Table 3), only *met-49* and *met-117* could be transduced by D3 lysates.

The phage preparations used for transductions were induced from D3 lysogens of strain PAO by irradiation with UV light. We found that phage progeny collected after induction of a lysogen transduced at frequencies of 10- to 100-fold higher than phage prepared from lytic infections of sensitive strains. Such enhanced production of transducing particles by induction of lysogenic cells is characteristic of specialized transducing phages (16).

At least one transductant colony was chosen at random from each of 26 separate transductions and tested for lysogeny by the methods of Miller et al. (19). All transductants tested had become lysogenic. In three separate experiments, RM45 transductants which had been transduced to methionine prototrophy by D3 grown on PAO1 were induced by exposure to UV light to release phage. The D3 released was then used to transduce RM45 to prototrophy. The transduction frequency in one of the three instances was increased by a factor of 10^3, indicating that high-frequency transducing (HFT) lysates of this phage can be produced. Thus when D3 transduces a cell, it usually converts that cell to a lysogen, and the newly formed lysogens can be used to produce HFT lysates by methods similar to those used to produce HFT lysates of phage lambda (17).

Calhoun and Feary (1) found *met-117* and *met-49* to be in unlinked transductional groups when phage F116 was used as the transducing vector. We confirmed that these markers are not cotransducible by F116. In addition, we were unable to detect cotransduction of *met-49* with *met-117* by D3. We conclude that *met-49* and *met-117* are not tightly linked.

Map location of *met-117* and *met-49*. Since neither D3 nor F116 will cotransduce *met-117* and *met-49*, we wanted to determine the location of these alleles on the *P. aeruginosa* PAO genetic map (24). Through a series of conjugation experiments we were able to map *met-117* to about 27 min on the PAO chromosome (Fig. 1A). FP2 is a chromosome-mobilizing plasmid that transfers clockwise from 0 min on the *Pseudomonas* chromosome map (12). FP110 mobilizes counterclockwise from 26 min (23). In interrupted matings mediated by FP2, *met-117* was transferred before *met-28* and *trpC*, while *met-49* was not transferred at detectable levels (Fig. 1). FP110 did not transfer either *met-117* or *met-49* as early markers. This places *met-117* between the FP110 origin of transfer and the *met-28* locus, and *met-49* somewhere later than *trpC* (Fig. 2). PAO1000 is an Hfr donor strain which mobilizes the chromosome clockwise from the *trpA,B* gene cluster (7). PAO1000 did not mobilize *met-117*, indicating that *met-117* lies between the FP110 origin of transfer and the PAO1000 origin, or at about 26 min on the *P. aeruginosa* map. PAO1000 did not transfer *met-49*.

The *met-49* allele has been located in the mid-to-late

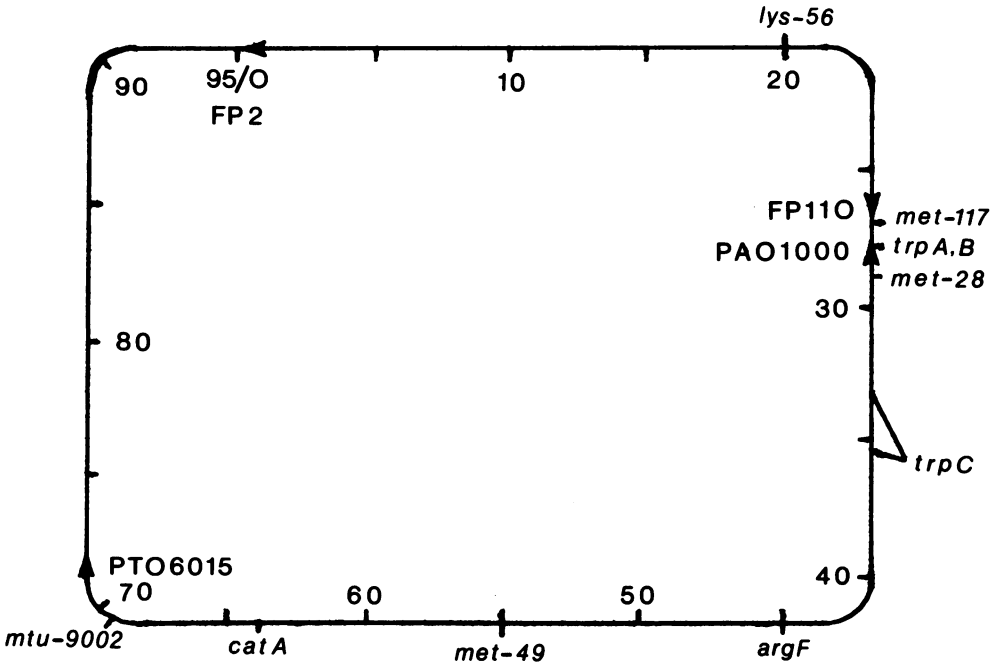

FIG. 2. Genetic map of *P. aeruginosa* PAO showing locations of *met-49* and *met-117* relative to previously mapped genes. Locations of previously mapped genes are from Royle et al. (24) and Früh et al. (4).

TABLE 4. Transductional complementation of *met-49* and *met-9011*

Donor strain[a]	Transductants/10^8 PFU with recipient strain[b]:		
	RM40 (*met-28*)	RM45 (*met-49*)	RM242 (*met-9011*)
RM1 (prototrophic)	2.5	1.2	0.9
RM45 (*met-49*)	11.0	<0.1	<0.1
RM242 (*met-9011*)	2.1	<0.1	<0.1

[a] Strains on which F116L lysates were prepared.
[b] Strains transduced by F116L to methionine prototrophy.

region of the map by conjugating with the donor PTO6015 (Fig. 1B). PTO6015 is an Hfr donor which was constructed by D. Haas by the insertion of the chromosome-mobilizing plasmid pME134 in a unique orientation at 72 min (personal communication). PTO6015 donates chromosome in a counterclockwise direction starting at 72 min on the PAO map (24). In interrupted matings between PTO6015 and RM268, we observed essentially complete transfer of *mtu-9002* and *catA1* at our earliest time point (data not shown). In other crosses (Fig. 1B), *met-49* was transferred while *argF* was not. Therefore *met-49* lies between *argF* (45 min) and *catA* (63 min). F116L transductional analysis (Table 4) indicated that *met-49* is very tightly linked to *met-9011*, which maps at 55 min (4), suggesting that *met-49* and *met-9011* are alleles of the same gene.

Site of integration of the D3 prophage. As the D3 prophage must be induced before significant levels of transduction of specific alleles can be observed, it seemed likely that the D3 prophage integrates near the loci which it transduces. We tested this possibility by carrying out uninterrupted matings between lysogenic donor strains and recipients which were resistant to the phage under study. The resistance of the recipient strain to the phage ensured that lysogenization could not take place by adsorption of free phage which might be released by the lysogenic donor. Lysogenization was restricted to acquisition of the phage through transfer of the prophage during conjugation. Preliminary tests for zygotic induction of the D3 prophage indicated that this phenomenon did not take place under the conditions used in our mating experiments (data not shown).

In FP2-mediated conjugations, the D3 prophage was coinherited with *met-117*. Donor strain RM17 was mixed with recipient RM2063, and methionine prototrophs were selected. Of these, 317 were streaked out for single colonies and tested for acquisition of D3 prophage. Of the recipients that had acquired *met-117*, 315 (99%) had concurrently inherited the D3 prophage. Similar experiments were done using RM172 as donor and selecting for various other genetic markers, including *met-49* (Fig. 3). The D3 prophage was found to be coinherited with high frequency with genes in the 20- to 30-min region of the genetic map and with *met-49*.

DISCUSSION

This is the first reported example of a specialized transducing phage in *P. aeruginosa* PAO. The two genes which it transduces are widely separated on the chromosome, and the D3 prophage integrates adjacent to both these genes. Carey and Krishnapillai (2) have reported that phage H90 integrates into the *P. aeruginosa* PAO chromosome at 7 min. They were unable to demonstrate specialized transduction by this virus.

Many of the properties of phage D3 are similar to those of other specialized transducing phages (5, 16). The establishment of lysogeny followed by the induction of established prophage is necessary for the production of transducing particles. Like coliphage lambda, this induction appears to require that the host have a Rec^+ phenotype and suggests that a protein similar to the *recA* gene product of *Escherichia*

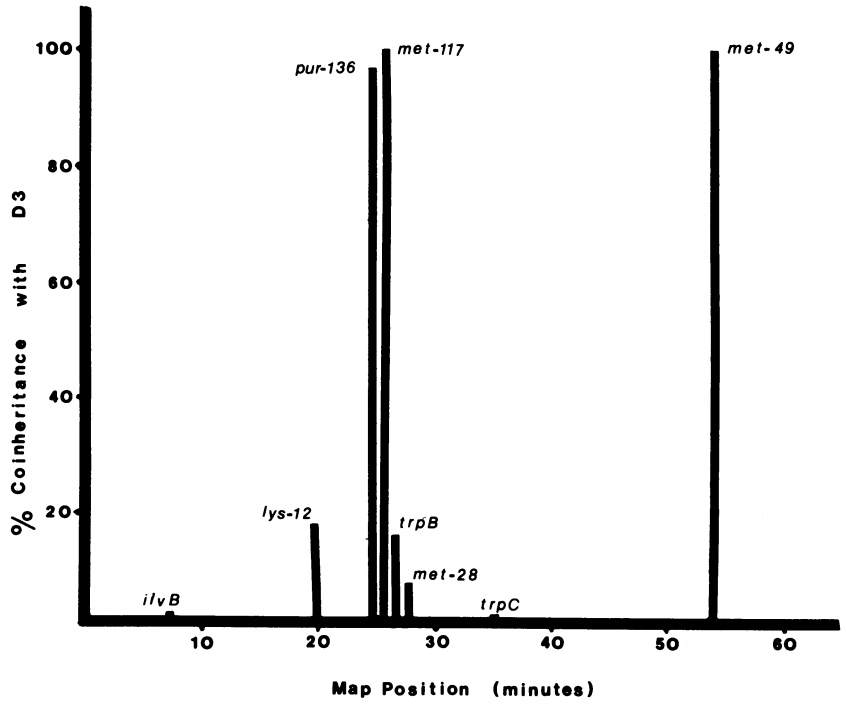

FIG. 3. Coinheritance of selected alleles with the D3 prophage. See the text for experimental details.

coli is required for UV-light-stimulated induction (15). This dependence is further emphasized by the fact that several mutations which reduce the recombinational proficiency of *P. aeruginosa* also reduce the ability of phage D3 to establish lysogeny (8, 18, 25). The D3 prophage integrates into the host chromosome at a minimum of two sites which are closely linked to the loci which it transduces. Transductants appear to be lysogens of prophage D3, and HFT lysates can be produced from transduced clones. These data suggest that transducing particles contain both bacterial and phage DNA and that the insertion of the bacterial fragment does not eliminate essential phage genes (i.e., transducing particles are not defective). The identification of a specialized transducing phage of *P. aeruginosa* provides an opportunity to address a myriad of questions concerning the genetics and biochemistry of this organism.

ACKNOWLEDGMENTS

We thank D. H. Calhoun, G. A. Jacoby, D. Haas, and B. W. Holloway for the kind gifts of many of the strains used in this study.

This work was supported in part by a grant from the Pott's Fund of the Stritch School of Medicine, Loyola University of Chicago, and by Public Health Service grant AI-12759 from the National Institute of Allergy and Infectious Disease.

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