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Prophage F116: Evidence for Extrachromosomal Location in *Pseudomonas aeruginosa* Strain PAO

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F116 is a temperate-generalized transducing phage of *Pseudomonas aeruginosa*. Genetic evidence leads to the conclusion that F116 prophage DNA is maintained extrachromosomally as a plasmid. Preliminary physical evidence is presented to support this hypothesis.

F116, originally identified in 1960 (6), is a generalized transducing DNA phage of *Pseudomonas aeruginosa*. This phage has been widely used for transductional analysis of the *P. aeruginosa* genome (12), yet little is known about the phage itself. In a previous communication (9), data was presented on the structure of the virion of this phage and the size, structure, molecular weight, and density of its DNA. This paper presents data on the localization of the prophage within the lysogenized cell.

Several strains of *P. aeruginosa* were used in these studies. Their phenotypic and genotypic characteristics are presented in Table 1. An F116 lysate was obtained from B. W. Holloway, and a clear plaque mutant (F116c) was selected from the lysate.

We first assumed that the prophage of F116 was integrated into the host chromosome. In an attempt to localize the integration point, we carried out uninterrupted matings between streptomycin-sensitive (Sm^s), lysogenic donors ($FP2^+$) and streptomycin-resistant (Sm^r), nonlysogenic, F116-resistant ($F116^r$) recipients (FP^-).

Matings were between donor cells growing in logarithmic phase and recipient cells in stationary phase. Stationary-phase cultures were obtained by overnight incubation at 37°C of unaerated inoculated Luria complete medium (L-broth, [12]). Logarithmic-phase cultures were obtained by inoculating a sample of stationary-phase culture into Luria complete medium and incubating with aeration at 37°C until three to four generations of growth had elapsed. Cell concentrations were followed with a Klett-Summerson colorimeter at 660 nm. Prior to mating, the cells of each strain were harvested by centrifugation. They were washed with and suspended in half-strength 56 medium (a pH 6.7 phosphate-buffered salts medium [10]). Concentrations of bacteria were adjusted to give a 1:1 ratio of the two types of cells. Mating pair formation was allowed to proceed for 15 min at 37°C before the mixture was diluted and plated on selective minimal medium. Minimal medium plates contained 1.5% agar, 0.1 M potassium phosphate buffer (pH 7.0), 3 mM sodium citrate, 0.8 mM $MgSO_4$, 15 mM $(NH_4)_2SO_4$, 0.4% glucose, and the appropriate supplements. Amino acids and nucleotides were present, when necessary, at a concentration of 50 μg/ml, and thiamine was used at a concentration of 0.2 μg/ml. Streptomycin (1 mg/ml) was used to contraselect the donor cells in all crosses. Streptomycin contraselection has the advantage of being bactericidal, which reduces the danger of carry-over of viable donor cells from the selective plates. The resistance of the recipient strain to F116 insured that lysogenization could not take place by adsorption of free phage in the mating medium and restricted the acquisition of the phage by the recipient cell to transfer of the prophage during the conjugation.

As a first step in mapping the F116 prophage, a cross was performed, the interpretability of which depended on the amount of prophage zygotic induction that followed conjugation. Lysogenic (JC3613) and nonlysogenic (JC9005) donor strains (otherwise isogenic) were mated with a recipient strain (JC8725), which carried several auxotrophic markers (see Table 1) located at intervals along the *P. aeruginosa* chromosome map (13). A comparison of recombination frequencies for each of these markers after uninterrupted matings was made. If F116 prophage is induced to lytic growth upon entering the repressor-free cytoplasm of the recipient cell, then the lysogenic donor should produce fewer recombinants for proximal and distal markers than the nonlysogenic donor. The results of these experiments indicate that, under

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TABLE 1. *Bacterial strains*

Strain	Sex	Genotype[a]									Prophage[a] (F116)	Response to infection[a]	Source or reference
		thi	*his*	*trp*	*lys*	*met*	*pyr*	*ser*	*pur*	*str*			
PAO1	FP⁻	+	+	+	+	+	+	+	+	+	–	S	ref. 5
PAO283	FP⁻	+	-3	-6	-56	-28	+	+	+	+	–	S	B. W. Holloway
PAO851	FP⁻	-1	-151	-150	+	+	B21	+	-66	+	–	R	ref. 13
JC3626	FP⁻	+	-3	-6	-56	-28	+	+	+	–	–	S	PAO283
JC3633	FP⁻	+	-3	-6	-56	-28	+	+	+	–	–	R	JC3626
JC8725	FP⁻	-1	-151	-150	+	+	B21	+	-66	–	–	R	PAO851
JC9006	FP⁻	+	+	+	+	+	+	+	-600	+	–	S	ref. 12
JC9013	FP⁻	+	+	+	+	+	+	+	-600	+	+	I	JC9006
PAO31	FP2⁺	+	+	+	+	+	+	-3	+	+	–	S	B. W. Holloway
JC3613	FP2⁺	+	+	+	+	+	+	+	-600	+	+	I	JC9005
JC3637	FP2⁺	+	+	+	+	+	+	-3	+	+	+	I	PAO31
JC9005	FP2⁺	+	+	+	+	+	+	+	-600	+	–	S	JC9006

[a] Genotype symbols follow the conventions recommended by Demerec et al. (4). The three letter portion of the symbol heads each column. A hyphen followed by an allele number completes the symbol if there is a mutation, and a plus sign completes the symbol if there is no mutation. In the case of *str* mutations, no allele numbers for mutations have yet been assigned to our knowledge; hence, the symbols are completed with a minus sign, indicating the presence of an unspecified mutation. As recommended by Demerec et al., when the cistron in which a mutation lies is specified, the hyphen is replaced by an upper case letter, as in the case of the *pyr* mutations. Phenotype abbreviations are employed to refer to the effects of a mutation. Mutations affecting biosynthetic pathways leading to end-product requirements are generally abbreviated in a way closely related to the gene symbol. There are two exceptions: *pyrB21* leads to a requirement for uracil (Ura⁻) and *pur-66* leads to a requirement for adenine (Ade⁻). Other abbreviations employed are as follows: his, histidine; lys, lysine; met, methionine; ser, serine; thi, thiamine; trp, tryptophane; str, streptomycin; S, sensitivity; R, resistance; and I, immunity. When used with phenotype abbreviations, the plus sign indicates ability to biosynthesize, and the minus sign indicates inability to biosynthesize: hence a growth factor requirement. Prophage are symbolized by including the name of the phage in parentheses. In this case, the plus sign indicates presence and the minus sign absence of the prophage.

the conditions used, there is only a small amount of zygotic induction. For each of the markers tested, which enter between 5 (*thi-1*⁺) and 29 min (*pyrB21*⁺), the difference in the number of recombinants was minimal. The variation in number of recombinants produced ranges from as little as a 1.1-fold difference for *trp-150*⁺, which enters at 23 min, to never greater than a 1.9-fold difference, observed for a marker which enters at 29 min (*pyrB21*⁺). These results encouraged us to attempt to determine the map location of phage F116 through matings similar to those used to determine zygotic induction. Matings between lysogenic (JC3613, JC3637) and nonlysogenic (JC9005, PAO31) strains and two nonlysogenic F116ʳ recipients (JC8725, JC3633) containing several auxotrophic markers (see Table 1) were carried out. Transconjugants were selected for each donor marker with streptomycin as the contraselecting agent. Those inheriting donor prototrophic markers were selected on appropriately supplemented minimal media and were purified on the same minimal medium in the presence of streptomycin.

Purified transconjugants were scored for coinheritance of the other markers by replica plating and for the lysogeny/nonlysogeny phenotype by testing for spontaneous release of phage on an indicator strain (PAO1). This was accomplished by replica plating onto a Luria agar plate seeded with PAO1. After growth at 37°C, lysogenic transconjugants showed a clear zone around the patch, due to release of infective phage particles. Release of phage was confirmed by growing small cultures of putative lysogens in Luria broth and plating appropriate dilutions of the culture medium to determine the titer of free phage. Transconjugants producing culture supernatants with titers of at least 10^5 PFU/ml were regarded as lysogenic.

Two hundred transconjugants from the cross of the F116ʳ recipients with the nonlysogenic donor were purified and tested for phage resistance by replica-plating patches onto a lawn of phage. No F116ˢ transconjugants were found, indicating that the wild-type marker for sensitivity is probably transferred late in an FP2-mediated mating. The frequency of coinheritance of lysogeny with each of the donor prototrophic markers was determined in the crosses of the F116ʳ recipients with the lysogenic donor. It was virtually 100% (Table 2) in all cases. Remembering that a similar phenomenon had been observed with the inheritance of the F2 plasmid in *E. coli* (1), we hypothesized that F116 prophage was also a plasmid.

P. aeruginosa, like *Escherichia coli*, can transfer its sex factor during a conjugal mating without the transfer of chromosomal material. The transmission of FP2 can be easily detected, because this plasmid carries the genetic information for resistance to mercury ion (Hgʳ) (8). To test for the conjugal transfer of F116 pro-

phage independent of chromosomal transfer, JC3613 (an FP2$^+$ Sm^s F116 lysogen) was crossed to JC8725 (FP$^-$ Sm^r F116^r nonlysogen), and Hg^r [Sm^r] transconjugants were selected. These were tested for lysogeny, as described above, and for inheritance of chromosomal markers. Twenty-five percent (50/200 transconjugants in two experiments) of the transconjugants, which had inherited FP2 but no donor chromosomal markers, were found to be F116 lysogens. As in the experiments reported in Table 2, virtually all transconjugants selected for inheritance of chromosomal markers were lysogens, but less than 2% had inherited the mercury resistance carried by FP2. This is further evidence for transfer of F116 independently of both the bacterial chromosome and FP2.

To support the genetic evidence for the plasmid nature of the F116 prophage, CsCl density gradients of DNA from lysogens and nonlysogens were compared. JC9006 is an adenine-requiring derivative of *P. aeruginosa* PAO1, and JC9013 is an F116 lysogen of JC9006. Ten-milliliter cultures of each of these strains were labeled with 50 μCi of [2-^{3}H]adenine (specific activity, 22 Ci/nmol; Schwartz/Mann). The cells were lysed, and the crude lysates were centrifuged to equilibrium in a CsCl gradient. The method for lysis and centrifugation was essentially that of Pemberton and Clark (4). In addition to treatment with RNase, the extracts were also treated with auto-digested Pronase (3) at a concentration of 0.5 mg of Pronase per ml of sample solution. This was done to prevent macromolecular aggregation. Figure 1 is representative of the data obtained in several repetitions of the experiment. A small satellite band, slightly lower in density (ρ = 1.720 g/cm^3) than the main band (ρ = 1.726 g/cm^3), is present in the extract of the lysogen and is absent in that

TABLE 2. *Coinheritance of F116 lysogeny with selected chromosomal markers*

Donor	Recipient	Selected marker[a] (% coinheritance)[b]							
		thi-1$^+$ (5)	*his-151*$^+$ (7)	*his-3*$^+$ (12)	*lys-56*$^+$ (18)	*trp-150*$^+$ (23)	*met-28*$^+$ (28)	*pryB*$^+$ (29)	*trp-6*$^+$ (32)
JC3613	JC8725	100	98	–[c]	–	100	–	99	–
JC3637	JC3633	–	–	100	100	–	100	–	100

[a] Parenthetical numbers placed after the genotype designation indicate its map position in minutes.

[b] Each percentage is the average of two experiments for a total of 200 selected exconjugants for each marker.

[c] The symbol (–) indicates that the recipient is prototrophic for the marker in question.

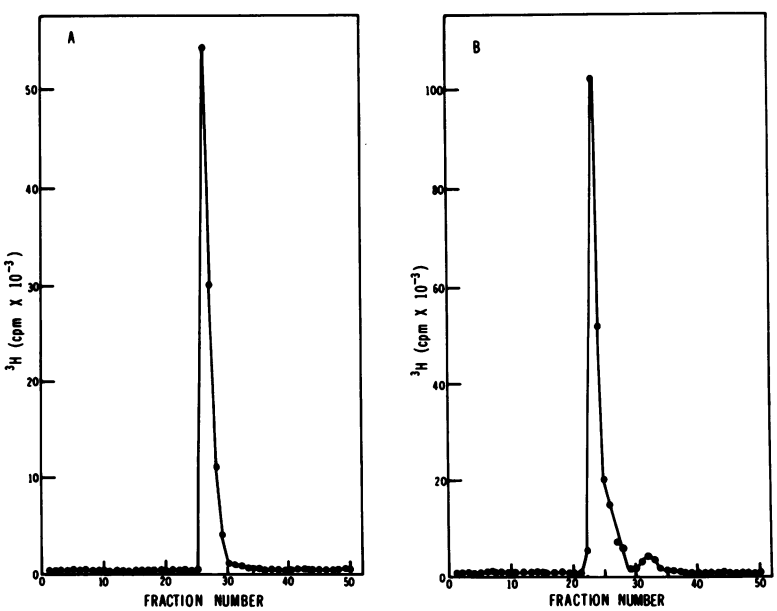

FIG. 1. *(A) CsCl gradient analysis of lysates of JC9006 and (B) JC9013. Cultures (10 ml) were grown in the presence of [2-^{3}H]adenine to the log phase. The lysates were prepared as described in the text and centrifuged for 60 h at 15°C at 44,000 rpm in a Spinco fixed-angle rotor type 50. Density increases to the left.*

of the nonlysogen. This agrees well with the densities of F116 virion DNA and that of *P. aeruginosa* DNA, respectively (9). We thus conclude that F116 prophage DNA is extrachromosomal. In addition, the satellite band represents 2 to 3% of the radioactivity present in the chromosomal peak. Since the virion DNA of phage F116 has a molecular weight of 3.8×10^7 (9), or approximately 2% of the molecular weight of the host chromosome (2.1×10^9, [11]), there may be only one copy of the prophage per chromosome. This preliminary physical characterization is in good agreement with the genetic evidence for an extrachromosomal location of prophage F116 in lysogens of strain PAO.

Several features of the behavior of F116 prophage seem worthy of future attention, in that they are similar to or different from the behavior of *E. coli* prophages known to be inherited as plasmids. The first noteworthy feature is the apparent inefficiency with which the F116 prophage is induced when transferred to a zygote, as evidenced by the high frequency of prophage inheritance among transconjugants inheriting donor chromosomal markers, and the small difference in the numbers of those transconjugants sired by lysogenic and nonlysogenic donors. In *E. coli*, lambda-prophage is efficiently induced by conjugational transfer to a nonlysogen (7). Although wild-type lambda-prophage is integrated with the *E. coli* chromosome, a mutant of lambda establishes lysogeny as a plasmid (15). The second noteworthy behavioral feature of F116 prophage is the apparent high efficiency prophage mobilization by the conjugative plasmid FP2; the high frequency of lysogens among transconjugants selected for their inheritance of FP2 supports this. One suspects from the data of Boice and Luria (2) that the mobilization of a *lac*$^+$-transducing derivative of P1 by F would have been less than one-tenth the frequency of the mobilization of F116 by FP2. A third feature of the F116 prophage worth comparing with *E. coli* prophages is the low lysogenization frequency of F116 characteristic of some recombination-deficient mutants of *P. aeruginosa* (16). This low lysogenization frequency of F116 is reminiscent of the reduced lysogenization frequency of a chloramphenicol-resistance-transducing derivative of P1 (14) produced by a *recA* mutation.

Study of such specific examples of F116 prophage behavior will lead to a more detailed understanding of the physiology of *P. aeruginosa* as compared with that of *E. coli*.

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