Prophage Induction of Noninducible Coliphage 186

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Coliphage 186 has been regarded as a member of the noninducible group of coliphages. Evidence that prophage 186 is induced by ultraviolet irradiation or by treatment with nalidixic acid or mitomycin C is now presented. The phage yields were similar to those from lysogens of the inducible phage lambda, and the induction required a *recA*⁺ host. A noninducible mutant of 186 was isolated from its heat-inducible derivative, 186*cIts*, that was no longer inducible by ultraviolet irradiation but remained heat inducible. That zygotic induction of 186 after transfer from a lysogenic male to a non-lysogenic recipient did not occur is indicated by the following findings: (i) there was only a slight increase in phage titer; (ii) similar levels of recombinants were obtained for markers adjacent or distal to the phage integration site, whether the recipient was lysogenic or not, and there was no effect on the gradient of marker transfer; (iii) lysogenic recombinants were readily found and the co-transfer of 186 with adjacent markers was the same to lysogenic or non-lysogenic recipients. Thus, 186 formed an inducible prophage that did not display zygotic induction. Nevertheless, it shared many properties with the noninducible phage P2 as outlined in the discussion.

Coliphage λ is the classic example of an inducible phage. Other inducible coliphages described by Jacob and Wollman (14) all recombine with λ and form prophages inducible by UV irradiation and on conjugation into a nonimmune recipient (zygotic induction). The members of the group of noninducible coliphages isolated by Jacob and Wollman (14) do not recombine with λ and form prophages which are neither inducible by UV irradiation nor zygotically inducible. Coliphage P2 also cannot be induced and has been described as the prototype of the noninducible phages (3). Coliphage 186 was placed in the noninducible group as it neither recombined with λ nor exhibited zygotic induction (14). UV induction of prophage 186 was not tested.

Coliphage 186 resembles the T even phages in morphology (2), and its DNA has a molecular weight of 2×10^7 (26). It integrates into the host chromosome near *pheA* (30). Baldwin et al. (2) isolated a temperature-inducible mutant of 186, designated 186*p* by them, which we shall refer to as 186*cIts*. This paper will show that prophage 186 is also UV inducible like λ , but that it is not zygotically inducible.

MATERIALS AND METHODS

Bacteriophages. The noninducible phages 18, 299, and W were obtained as lysogens from R. L. Baldwin, and P2.K from B. Kelly. Phage 186 and the tempera-

ture-sensitive clear plaque mutant 186*cIts* (designation changed by us from 186*p* [2] to conform with other clear plaque nomenclature) were from R. L. Baldwin. Inducible phages 434, λ , and its temperature-inducible mutant λ ind*cIts*857 were from D. S. Hogness. The noninducible mutant λ ind was from A. J. Pittard, and the host range mutant λ c*Its*857h80 was from N. C. Franklin. Phage T6 was from P. Reeves.

Bacteria. The *Escherichia coli* strains used in this work are described in Table 1. Lysogens of many of these strains were constructed with 186, or other phage described above, as prophage.

Media. Tryptone broth (TB) containing (per liter) 10 g of tryptone (Difco) and 5 g of NaCl was used for growth of indicator bacteria and of strains for UV induction experiments. L broth containing (per liter) 10 g of tryptone (Difco), 5 g of yeast extract (Difco), and 10 g of NaCl was supplemented with 0.1% glucose (LGB) for conjugation and other experiments. Where phage P2 was involved, LGB was supplemented with 2 mM CaCl₂ (LGCB). H-1 buffer salts solution (16) was used for diluting and resuspending bacteria. Phage were diluted in TM containing 0.01 M Tris, pH 7.0, and 0.01 M MgSO₄. The minimal medium used was half-strength medium 56 of Monod et al. (21), supplemented with glucose (0.2%) as carbon source and with amino acid and vitamin requirements as appropriate. The above broths and minimal medium were solidified with 7 g of agar (Difco) per liter for overlays and 15 g of agar per liter for plates.

Sources of other chemicals were as follows: streptomycin sulphate (Drug Houses of Australia Ltd., Adelaide, South Australia), nalidixic acid (gift of Winthrop Laboratories, Ermington, New South

TABLE 1. *E. coli* strains utilized^a

Strain	Sex ^b	Relevant genotype	Source
<i>E. coli</i> K-12			
152	F ⁻	<i>recA56 gal str^r</i>	D. S. Hogness
594	F ⁻	<i>galK galT str^r</i>	D. S. Hogness
AB259	HfrH	<i>thi str^a λ⁻</i>	P. Reeves
AB1133	F ⁻	<i>thr leu pro his argA thi str^r</i>	P. Reeves
AB2463	F ⁻	<i>thr leu pro his arg thi str^r recA13</i>	J. Davison
AB2528	HfrO-13	<i>leu thi ilv supM str^r tsx^a (λ)</i>	A. J. Pittard
AT2092	F ⁻	<i>his purF pheA thi str^r tsx^r (Mul)</i>	A. J. Pittard
C600	F ⁻	<i>thr leu thi tonA supE</i>	D. S. Hogness
DM455	F ⁻	<i>galK galT str^r recA99</i>	B. Rolfe
JC2926	F ⁻	<i>thr leu pro his arg str^r recA13 supE</i>	A. J. Clark
JP190	F ⁻	<i>tyrA thi ilvD metB argH str^r tsx^r λ^r</i>	A. J. Pittard
JP264	F ⁻	<i>his thi ilvD metB argH str^r tsx^r λ^r nal^r</i>	A. J. Pittard
KL16	Hfr	<i>thi str^a λ⁻</i>	P. Reeves
<i>E. coli</i> C			
C-436	F ⁻	<i>T1, try his arg str^r</i>	R. Calendar
C-1055	F ⁺	<i>thr leu xan his str^r</i>	M. Mandel

^a The genetic symbols are those used by Taylor and Trotter (25) and Wiman et al. (28).

^b The origins of the Hfr strains are shown in Fig. 2.

Wales), mitomycin C (Kyowa Hakko Ltd., Tokyo, Japan), and NNG (*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, Aldrich Chemical Co., Milwaukee, Wisc.).

Assay of bacteria and phage. Bacteria were diluted in H-1 and spread on L plates to assay colony formers per milliliter. Phage were diluted in TM or in H-1 if infectious centers were involved, and 0.1-ml samples were mixed with 0.2 ml of log phase (1.5 absorbancy units at 600 nm [A_{600} 1.5]) indicator bacteria. Strain C600 was the standard phage indicator, with 594 and AB1133 being used where a streptomycin or phage T6-resistant indicator, respectively, was required. *E. coli* C-1055 grown in LGCB was the indicator used for P2. Preadsorption was allowed for 15 min, if free phage only were being assayed, before plating in an overlay of TB soft agar on TB (LGCB for P2) plates. After overnight incubation at 37 or 30 C, plaques were counted.

Construction of lysogens. Lysogens were prepared from the turbid centers of single plaques of the phage on the particular strain as indicator. They were purified through at least three single colony isolations, lysogens being identified throughout by phage production on appropriate indicators, or by immunity to superinfection.

Heat induction. Lysogens of heat-inducible phage mutants were grown to A_{600} 1.0 at 30 C, shaken at 45 C for 10 min, and then at 37 C till lysis.

UV induction. Lysogenic cultures grown at 30 C in TB to A_{600} 0.8 to 1.0 were centrifuged and resuspended in H-1 and UV irradiated with swirling in a layer less than 1-mm deep for 50 s, 50 cm from a General Electric 15-W germicidal lamp (dose 760 ergs/mm²). One-tenth volume of 10 times concentrated TB was added and the resultant culture was shaken at 30 or 37 C till lysis.

NNG mutagenesis. C600 (186*cIts*) was grown at 30 C to A_{600} 0.8. The culture was transferred to 40 C

for 25 min, then NNG (8 µg/ml) was added. After a further 15 min at 40 C, the culture was diluted 10³-fold in fresh broth and allowed to lyse at 40 C yielding mutagenised phage.

Conjugation. Conditions were as described by Adelberg and Burns (1), save that lysogenic donors and/or recipients were involved. Cultures were grown in LGB to A_{600} 0.8, then washed and resuspended at 20 C in fresh LGB to reduce the free phage concentration. Hfr and F⁻ bacteria were mixed in a ratio of 1:10 to 1:20, respectively, in the presence of rabbit antiserum to the phage concerned ($K = 1.5$ per min for λ, $K = 3.6$ min⁻¹ for 186) and swirled very gently in a flask with a capacity at least 20 times the volume of the mating mixture. After mating, samples were diluted into H-1 containing streptomycin (200 µg/ml) or added to phage T6 as described by Hayes (13) to kill the donor cells. Dilutions were spread on minimal plates to select recombinants or plated immediately with appropriate indicator bacteria on TB plates (containing streptomycin, if appropriate) to assay phage activity. Isolated recombinants were tested for 186 lysogeny by stabbing them with a sterile toothpick into plates seeded with 594 indicator bacteria.

RESULTS

Immunity and host range. Phage 186 has an immunity different from those of the λ-related inducible phages λ, 434, 21 (14), and from those of the noninducible phages 18, W, 62, 299 (12), P2, P3, and P2Hydis (4). We confirmed that many of these phages, as well as φ80, P1, and Mu1, were heteroimmune to 186.

E. coli K-12 was the normal host for phage 186, and the phage was active on a large number of K-12 strains, including standard phage indicators C600, 594, the S26 derived set of

isogenic suppressor strains (12), as well as on male strains such as AB259 and KL16. Phage 186 did not form plaques on *E. coli* C strains. However, C strains can host the 186 lytic cycle since *E. coli* C (186), isolated from among *his*⁺ recombinants after conjugation between HfrKL16 (186) and C-436 F-*his*⁻ (or C-1055 F-*his*⁻), yielded 186 phage active on K-12 indicator bacteria. Such C (186) lysogens remained resistant to a virulent mutant of 186. Phage 186 was found not to adsorb to C strains, which explains the inability of phage 186 to plaque on *E. coli* C.

On *recA* strains, 186, P2, and λ , all formed turbid plaques. On *recB* or *recAB* strains, P2 forms clear plaques (18), whereas 186 forms turbid plaques like λ . In further contrast to P2, 186 lysogens permit λ to plaque and thus do not exhibit the Old⁺ phenotype displayed by P2 lysogens (18).

Bacterial mutants resistant to phage 186 are readily isolated and fall into two classes. For example, phage 186-resistant derivatives of JP264 were selected as the survivors of massive infection by 186*cIts* at 37 C. Of 30 survivors that proved to be 186 resistant, five were also resistant to phage 299, defining two classes (Table 2). Members of class I were resistant to phages 186 and W, but sensitive to phages 18, 299, and P2, which is a pattern similar to that of the 186^r strain isolated by Jacob and Wollman (14). Members of class II plated none of the noninducible group of phages, a pattern shared by the *rep* mutants (7) isolated as resistant to ϕ X174, and by a mutant isolated as resistant to noninducible phage 62 by Jacob and Wollman (14). For a sample member of each class, the defect

TABLE 2. Sensitivity of JP264- and 186-resistant derivatives of JP264 to other coliphages^a

Phage spot	Indicator bacteria		
	JP264 ^b	JP264 186 ^r (class I)	JP264 186 ^r (class II)
T6	-	-	-
λ	-	-	-
λ cIts857h80	+	+	+
434	+	+	+
186	+	-	-
186 <i>cIts</i>	+	-	-
W	+	-	-
18	+	+	-
299	+	+	-
P2.K	+	+	-

^a Tested by spotting phage stocks (10⁷ PFU/ml) on indicator lawns and incubating overnight at 37 C. +, Phage activity; -, no activity.

^b JP264 is itself resistant to λ and T6.

was shown to be a lack of 186 adsorption. In addition, prophage 186*cIts* was introduced by conjugation at 30 C from HfrKL16 (186*cIts*). His⁺ recombinants were selected and, of these, 50% carried the prophage, similar co-transfer to that reported previously (30). Such recombinants retained the phage sensitivity pattern of the particular 186-resistant parent and produced similar levels of free phage to that from the 186-sensitive lysogen on heat induction. The phenotype of class I and class II 186^r mutants appears therefore to reflect an adsorption defect rather than replication inhibition.

UV induction. The changes in absorbance at 600 nm following UV irradiation of C600 lysogens of λ , 186, and 186*cIts* are shown in Fig. 1 for post-irradiation incubation at 37 C. There was only a slight drop in absorbance when cultures were grown after irradiation of the 186 lysogen compared with that for the λ lysogen. Incubation at 37 C alone was sufficient to induce the 186*cIts* lysogen, but when this was irradiated, the drop in absorbance was much less and resembled more the behavior of the 186 lysogen. Similar results were obtained when the post-irradiation incubation was at 30 C, except that the 186*cIts* lysogen was not heat induced and, without irradiation, showed no lysis.

Although 186 lysogens showed little lysis in response to UV irradiation, the increase in

FIG. 1. The changes in absorbance (600 nm) following UV irradiation of C600 lysogens of phage λ (●), phage 186 (▲), and phage 186*cIts* (■) over 180 min of post-irradiation incubation at 37 C. The open symbols represent the non-irradiated controls.

phage titer indicates that the 186 prophage was UV inducible (Table 3). Prophages λ and 186 showed a 10^3 - to 10^4 -fold increase in phage titer. The titers for induced and noninduced cultures of the 186 lysogen were greater when the incubation was at 30 C rather than 37 C. With 37 C incubation, prophage 186*clts* showed heat induction, but at 30 C, similar UV induction to that for 186 was obtained. In contrast, lysogens of P2, 18, 299, and W showed no elevation in phage titer in response to UV irradiation. In fact, they yielded similar titers after 30 or 37 C incubation to those from the mutant noninducible prophage λ ind*clts* after 30 C incubation.

Since 186 has always been classified as noninducible, strains other than C600 were investigated, and 186 lysogens of strains 594, W3805, and CR34, among others, were found to be UV inducible. Phage 186 from another source (186*am* mutants from A. D. Kaiser) was checked and found to form an UV inducible prophage.

Induction in a *recA* host. From turbid plaques on *recA* indicator bacteria, *recA* lysogens of P2, 186, and λ were isolated and investigated. Like *recA* (λ) (5, 11), *recA* (186) produced low levels of free phage either spontaneously or following UV irradiation compared with the *recA*⁺ lysogen (Table 4). The majority of phage produced spontaneously were clear

plaque mutants. Irradiation greatly reduced the number of clear plaques, presumably through killing many of the cells which were to provide these mutants, while causing a five- to sixfold increase in the number of turbid plaques, which could be attributed to either a small amount of induction or to the presence of a low level of *recA*⁺ revertants in the culture. A larger dose of UV reduced both the clear and turbid plaque titers. However, heat induction of 186*clts* prophage was successful in the *recA* host AB2463.

Noninducible prophages in *recA* hosts were also investigated. The level of free phage present in the supernatant of a *recA* (P2) culture was the same as that for the *recA*⁺ (P2) culture, and the phage formed turbid plaques. In contrast, the situation for λ ind is identical with that of its parent λ ind⁺ (5, 11) in that the spontaneous titer was greatly reduced in the *recA* lysogen, and the majority of plaques produced were clear (Table 4).

Induction by nalidixic acid. Following treatment with 12.5 μ g of nalidixic acid per ml, lysogens of both λ and 186 showed about a fivefold increase in phage titer (Table 5), which is similar to the induction found for prophage λ by Cowlshaw and Ginoza (9) at this dose. Prophage 186 is not induced by nalidixic acid in a *nalA*^r strain (JP264), but it is UV inducible in this strain. The non-UV-inducible mutant λ ind is not induced by nalidixic acid, which confirms the suggestion of Cowlshaw and Ginoza (9) that nalidixic acid induction occurs by (indirectly) inactivating the repressor similarly to UV induction.

Induction by mitomycin C. Mitomycin C (1 μ g/ml) added to a freshly resuspended log phase (A_{600} 1.0) culture of C600 (186) in LGB caused no change in the absorbance of the culture relative to a nontreated control over a 240-min incubation at 30 C. However, phage titers at 240 min were 8.1×10^6 PFU/ml for the untreated culture and 1.5×10^{10} PFU/ml for the mitomycin-treated culture. An alternative method of induction with mitomycin was to dilute resuspended log phase cultures of C600 (λ) and C600 (186) and plate on TB plates with C600 indicator bacteria using a 2.5-ml layer of 0.7% TB agar containing 1 μ g of mitomycin C per ml. Under these conditions, 77% of the λ lysogenic bacteria scored as infectious centers, whereas 62% of the 186 lysogens did so.

Noninducible mutant of 186. Vegetative 186*clts* phage were mutagenised with NNG and then plated with C600 indicator bacteria on TB plates containing 0.2 μ g of mitomycin C per ml. After overnight incubation at 30 C under these conditions, 186*clts* gave clear plaques. From the

TABLE 3. UV induction of prophage 186 and other coliphages^a

Lysogen	Post-irradiation incubation temp	Phage titer (PFU/ml)	
		Non-irradiated culture	Irradiated culture
C600 (λ)	30	1.0×10^6	2.3×10^{10}
	37	1.4×10^6	2.8×10^{10}
C600 (λ ind <i>clts</i> 857)	30	1.0×10^4	5.0×10^4
	37	9.1×10^3	2.5×10^{10}
C600 (186)	30	1.5×10^7	3.2×10^{10}
	37	7.8×10^6	1.4×10^{10}
C600 (186 <i>clts</i>)	30	9.6×10^6	1.7×10^{10}
	37	1.9×10^{10}	9.0×10^9
C600 (P2)	30	4.1×10^5	3.7×10^5
	37	7.4×10^5	5.4×10^5
C600 (18)	30	1.0×10^4	1.5×10^4
	37	1.1×10^4	1.3×10^4
C600 (299)	30	2.0×10^4	3.2×10^4
	37	1.0×10^5	7.1×10^4
C600 (W)	30	2.6×10^5	1.5×10^5
	37	5.4×10^5	1.7×10^5

^a Cultures of the various lysogens were grown at 30 C in TB to A_{600} 1.2, divided, and one portion UV irradiated as described. Samples were assayed for phage titer at 180 min after irradiation when the post-irradiation incubation was at 37 C, and at 250 min when at 30 C. Phage assays were incubated overnight at 30 C, except for phage 18 which was assayed at 37 C since it did not form plaques at 30 C.

TABLE 4. Induction of *recA* lysogens of phage 186 and *λ*ind^a

Lysogen	Inducer	Phage titer (PFU/ml)	
		Turbid plaques	Clear plaques
DM455 (186)	None	3.0×10^2	4.5×10^3
JC2926 (186)	None	1.3×10^3	3.3×10^3
JC2926 (186)	UV 76ergs/mm ²	3.8×10^3	5.8×10^2
JC2926 (186)	UV 152ergs/mm ²	7.6×10^3	5.1×10^2
JC2926 (186)	UV 304ergs/mm ²	8.7×10^3	3.5×10^2
JC2926 (186)	UV 760ergs/mm ²	2.4×10^3	0.6×10^2
AB2463 (186 <i>clts</i>)	Heat	$1.0 \times 10^{*b}$	
152 (<i>λ</i> ind)	None	1.0×10^2	4.9×10^2

^a Cultures were induced as described. The phage titers shown are for samples after 180 min post-irradiation incubation at 37 C [250 min at 30 C for DM455 (186)]. For JC2926 (186), all doses of UV gave a bacterial survival of less than 10^{-4} .

^b Assayed at 30 C.

TABLE 5. Induction of prophages 186 and *λ* by nalidixic acid^a

Lysogen	Phage titer (PFU/ml)	
	Untreated culture	Nalidixic acid-treated culture
C600 (<i>λ</i>)	8.6×10^7	6.4×10^8
C600 (186)	9.3×10^7	6.9×10^8
JP190 (186)	1.5×10^8	7.4×10^8
JP264 (186)	3.2×10^8	3.9×10^8
AB259 (<i>λ</i> ind)	2.8×10^8	3.2×10^8

^a Lysogens were grown in LGB to A_{600} 0.5 and resuspended in H-1. One-tenth volume of $10 \times$ LGB and, to a portion of each, nalidixic acid (12.5 μ g/ml) were added, and the cultures were shaken at 37 C for 10 min. They were then diluted 100-fold into fresh LGB, shaken at 37 C for 180 min, and assayed for phage activity. The above titers include the dilution factor.

plating of the mutagenised stock, four turbid plaques were detected and purified—they are referred to as *tum* (turbid on mitomycin plates) mutants. C600 lysogens were prepared and investigated (Table 6). C600 (186*cltstum*1, 2, and 3) showed normal UV induction, except that the *tum*1 mutant did not plaque at 37 C. C600 (186*cltstum*4) was not UV inducible and either with or without UV about 30% of the phage produced formed clear plaques at 30 C (on TB plates without mitomycin). In contrast, supernatants of *tum*1, 2, or 3 lysogens contained only phage producing turbid plaques under these conditions. C600 (186*cltstum*4) remained heat inducible yielding a lysate with a titer of 3×10^{10} PFU/ml after 180 min incubation at 37 C following heat induction. The frequency of clear plaque formers at 30 C in this lysate was less than 10^{-3} .

Attempted zygotic induction of prophage

186. Using the conjugation conditions described above, and with phage antiserum to protect the phage-sensitive F⁻ strains, various crosses were performed in an attempt to show zygotic induction of prophage 186. The results appear in Table 7. The conditions allowed good zygotic induction of phage *λ* (lines 1 to 3), as exemplified by the great increase in phage titer where conjugation was to a non-lysogenic recipient (line 2), and by the reduced number of recombinants for an adjacent marker in this case, compared with the number obtained with a lysogenic recipient (line 3). In all cases with prophage 186 (lines 4 to 15), similar levels of recombinants were obtained for markers adjacent or distal to the phage integration site (Fig. 2), whether the recipient was non-lysogenic or a 186 lysogen. There may be an increase in phage titer on conjugation to the sensitive female compared with that from the male alone, but the level was always less than the transfer of the adjacent bacterial marker, and, in fact, simply represented subsequent spontaneous phage production on the assay plates in females that acquire the prophage through conjugation. There was never the great increase in phage titer and decrease in recombinants (especially for distal markers) as found with phage *λ*. Similarly, the potential zygotic induction of prophage 186 did not disturb the gradient of transfer of bacterial markers, which is markedly steepened by zygotic induction of phage *λ* (29). The gradient of transfer of *pheA*, *purF*, and *his* from donor KL16 (186) was the same whether the recipient was lysogenic or not (Table 8).

A further indication of zygotic induction was in the genotype of the recombinants obtained with a non-lysogenic recipient. For prophage *λ*, these recombinants were only very rarely lysogenic (data not shown, 15). However, for prophage 186, lysogenic clones were readily found.

TABLE 6. *Properties of 186cItstum mutants*

Property	Mutant				
	<i>tum1</i>	<i>tum2</i>	<i>tum3</i>	<i>tum4</i>	<i>tum</i> ⁺
Plaque on TB plate containing 0.2 μ g of mitomycin per ml at 30 C	Turbid	Turbid	Turbid	Turbid	Clear
Plaque on TB plate at 37 C	No plaques	Clear	Clear	Clear	Clear
Phage yield without UV ^a	2.6×10^6	1.0×10^6	1.0×10^6	3.7×10^5	9.6×10^6 ^b
Phage yield with UV ^a	1.2×10^9	1.1×10^9	5.3×10^8	3.7×10^5	1.7×10^{10} ^b

^a Cultures of C600 (186*cItstum*) lysogens were grown in TB at 30 C to A_{600} 1.2, divided, and one portion UV irradiated as described. Samples were assayed at 30 C on C600 for phage titer (PFU/ml) after 250 min post-irradiation incubation at 30 C.

^b Data from Table 3.

TABLE 7. *Zygotic induction of phages λ and 186^a*

Line	Donor	Recipient	Recombinants selected	Recombinants ^b per 100 Hfr cells	Plaques ^b per 100 Hfr cells
1	AB259 (λ)	Alone	<i>pro</i> ⁺ <i>str</i> ^r	<0.01	0.2
2	AB259 (λ)	AB1133	<i>pro</i> ⁺ <i>str</i> ^r	2.5	64
3	AB259 (λ)	AB1133 (λ)	<i>pro</i> ⁺ <i>str</i> ^r	6.2	
4	AB2528 (186)	Alone	<i>his</i> ⁺ <i>tsx</i> ^r	<0.01	0.9
5	AB2528 (186)	JP264	<i>his</i> ⁺ <i>tsx</i> ^r	1.2	1.4
6	AB2528 (186)	JP264 (186)	<i>his</i> ⁺ <i>tsx</i> ^r	1.5	
7	AB2528 (186)	Alone	<i>tyr</i> ⁺ <i>tsx</i> ^r	<0.01	0.8
8	AB2528 (186)	JP190	<i>tyr</i> ⁺ <i>tsx</i> ^r	2.6	1.4
9	AB2528 (186)	JP190 (186)	<i>tyr</i> ⁺ <i>tsx</i> ^r	2.7	
10	KL16 (186)	Alone	<i>tyr</i> ⁺ <i>str</i> ^r	<0.01	0.9
11	KL16 (186)	JP190	<i>tyr</i> ⁺ <i>str</i> ^r	19.1	5.6
12	KL16 (186)	JP190 (186)	<i>tyr</i> ⁺ <i>str</i> ^r	17.9	
13	KL16 (186 <i>cIts</i>) ^c	Alone	<i>tyr</i> ⁺ <i>str</i> ^r	<0.01	0.5
14	KL16 (186 <i>cIts</i>) ^c	JP190	<i>tyr</i> ⁺ <i>str</i> ^r	5.5	2.3
15	KL16 (186 <i>cIts</i>) ^c	JP190 (186)	<i>tyr</i> ⁺ <i>str</i> ^r	5.9	

^a All crosses as described.

^b Assayed after 60-min mating for lines 1 to 3 and 10 to 15, and after 90-min mating for lines 4 to 9.

^c Experiment performed at 30 C, all others at 37 C.

FIG. 2. *Escherichia coli* K-12 linkage map, based on that of Taylor and Trotter (25), showing origins of transfer of Hfr strains utilized.

For example, of the *tyr*⁺ recombinants of JP190 (Table 7, lines 8 and 11), approximately 90% now carry the 186 prophage (69/73 and 160/185, respectively). In addition, the lysogenic recombinants (4/4 tested) were UV inducible (method as detailed in Table 3; non-irradiated cultures, 1.1 to 1.3×10^7 ; irradiated cultures, 2.1 to 2.7×10^{10} PFU/ml), indicating that the lack of zygotic induction is not due to a permanent inability of the recombinants to allow 186 induction.

Perhaps the best demonstration of prophage 186 behaving as a normal bacterial marker is from the crosses involving donor KL16 (186*cIts*) (Table 7, lines 13 to 15). Where conjugation was to the non-lysogenic recipient JP190, 93% (153/165) of the *tyr*⁺ recombinants carried the 186*cIts* prophage. When conjugation was to JP190 (186), 89% (164/185) of the *tyr*⁺ recombinants carried 186*cIts*. Thus, the co-transfer of

TABLE 8. Effect of prophage 186 on the gradient of transfer of *pheA*, *purF*, and *his*^a

Donor	Recipient	<i>phe</i> ⁺ <i>str</i> ^r recombinants ^b per 100 Hfr cells	Analysis of <i>phe</i> ⁺ <i>str</i> ^r recombinants			
			<i>purF</i> ⁺		<i>his</i> ⁺	
			Fraction	%	Fraction	%
KL16	AT2092	33	52/89	58	25/89	28
KL16 (186)	AT2092 (186)	26	74/147	50	48/147	33
KL16 (186)	AT2092	30	121/231	52	72/231	31

^a Conjugation conditions as described.^b Selected after 60-min mating at 37 C.

186*clts* with *tyrA* was the same whether the recipient was lysogenic or not. Results obtained from interrupted mating experiments also indicated that 186 is not zygotically inducible (30). For the bacterial markers involved (*tyrA*, *pheA*, *purF*, and *his*), the same entry curves were obtained whether the female was lysogenic or not.

DISCUSSION

Although phage 186 shares some properties with the noninducible coliphages, it shares with λ the sensitivity of the prophage to induction by UV irradiation, mitomycin C, and nalidixic acid.

Both 186 and λ require a *recA*⁺ host to exhibit a normal level of UV induction or spontaneous phage production. In a *recA* host, both produce low levels of phage and many of these are clear plaque mutants. The noninducible phage, however, do not require *recA*⁺ product to exhibit their normal level of spontaneous phage production, as has also been found by others (Calendar and Laffler and Luria, as cited in 4). The noninducible mutant of λ , λ *ind*, does require the *recA*⁺ product for its normal spontaneous production. Thus, it appears that spontaneous phage production involves different mechanisms for the inducible phages (λ , λ *ind*) and for the noninducible phages (P2), and in this respect, 186 belongs to the inducible group.

Of the four 186*clts*-derived mutants isolated as turbid plaques on plates containing mitomycin at 30 C one proved to be not inducible by UV irradiation. The nature of the change in the other mutants is not known. Eshima, Fujii, and Horiuchi (10) found that all 32 λ mutants they isolated in this manner proved to be not inducible. The noninducible 186 mutant, 186*cltstum4*, is analogous to λ *indclts857* in that it is not UV inducible but remains heat inducible. It does not display the Nip⁺ phenotype of P2 (6) which would interfere with both UV and heat induction, again emphasizing the similarity between 186 and λ in induction properties. However, the 186*tum4* defect appears more extreme than the

λ *ind* defect, since many clear plaque mutants are found among phage produced spontaneously from the 186*cltstum4* lysogen. This is similar to the situation in *recA* lysogens of λ or 186 and may indicate that spontaneous phage production by C600 (186*cltstum4*) can only occur by mutation to a clear plaque type.

Prophage 186 is not zygotically inducible, unlike prophage λ and like prophage P2. The lack of zygotic induction of 186 is indicated by its overall behavior in conjugation from a lysogenic male to a non-lysogenic female. There is no great increase in phage titer (especially when compared with the transfer of an adjacent bacterial marker); recombinants for markers distal to the prophage are recovered and there is no change in the gradient of transfer of markers compared with crosses involving a non-lysogenic recipient. Prophage 186 behaves as a normal bacterial marker in its co-transfer with adjacent markers, and there is not the abortive zygotic induction as reported for P2 by Kelly (17). This is indicated by the similar co-transfer of male 186*clts* prophage to *tryA*⁺ recombinants, whether the recipient is non-lysogenic or lysogenic for 186.

There are compelling similarities between phages 186 and P2. Bertani and Bertani (4) have reviewed the properties of the noninducible coliphages, especially P2, and find that 186 is serologically related to P2, although to a lesser extent than are the other noninducible phages. The particle morphologies are similar (2, 4). The phage DNAs have similar molecular weights, share extensive homology (24; Young-husband and Inman, manuscript in preparation), and in fact recombine with one another to form hybrid phage (Bradley, Ong, and Egan, manuscript in preparation). The sticky ends of P2 and 186 DNA molecules are almost identical, differing in only 2 of 19 bases (22, 23), which allows the formation of mixed dimers (19). However, the ends are sufficiently different to be distinguishable by their melting profiles (20). Neither 186 nor P2 sticky ends will

cohere with those of phage λ (2, 19, 27). Replication of the 186 DNA is unidirectional (8) like P2, and unlike λ .

Although sharing the above properties, phages 186 and P2 are heteroimmune and differ in host range. The most significant difference is in their prophage induction behavior, for whereas 186 is non-zygotically inducible like P2, it is UV inducible like λ . This enigmatic lack of zygotic induction for an inducible phage will be the subject of further communication.

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