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Integration Site of Noninducible Coliphage 186

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From conjugational data, the attachment site for noninducible coliphage 186 (*att*186) was located between the origins of Hfr strains KL16 and KL98, and close to the *pheA* gene in *Escherichia coli* K-12. P1 transductions indicated that *att*186 lies at 51 min on the standard genetic map of *E. coli*, with the order *cysC-nalB-att*186-*pheA*. The presence of prophage 186 in the donor destroyed linkage between *nalB* and *pheA*, which is taken as evidence for the integration of the 186 prophage between these genes.

We have found that the prophage of coliphage 186 is induced by ultraviolet light but is not zygotically induced (23; Woods and Egan, *in preparation*). To explain this apparently contradictory behavior is a major aim of our program to study prophage induction, but our immediate interest here is to map the attachment site for this temperate phage (*att*186).

The loci of the attachment sites for a number of coliphages are known with various degrees of accuracy (9, 13, 18). For the inducible phages λ, 434, 82 (15), and φ80 (16), and noninducible phage P2 (location II; 4), the attachment sites are known accurately, integration of these prophages into the continuity of the bacterial chromosome being indicated by reduction in linkage between bacterial markers flanking the prophage. Further evidence demonstrating actual integration of the prophage, as proposed in the Campbell model of lysogeny (5), has been reviewed by Signer (17) and Gottesman and Weisberg (6).

Evidence is presented here for the location and integration of coliphage 186 at 51 min on the *Escherichia coli* K-12 linkage map (19). Jacob and Wollman in their original description of coliphage 186 placed *att*186 "on the segment of the bacterial linkage group carrying the maltose, mannitol and xylose characters" (10). During the course of this work, Abe and Tomizawa (1) reported limited P1 transduction data locating *att*186 close to 50 min.

MATERIALS AND METHODS

Bacterial strains. The strains used in this work are all derivatives of *E. coli* K-12 and are described in Table 1. Phage 186 lysogens, indicated (186), were isolated from the turbid centers of single plaques, and were purified by repeated single-colony isolation.

Bacteriophage strains. Phage 186 and the temperature-sensitive clear plaque mutant 186*p* (3) were obtained as lysogens from R. L. Baldwin, P1*vir* was obtained from A. J. Pittard, and T6 from P. Reeves.

Media. The complete medium used, L broth, contained (per liter) 10 g of tryptone (Difco), 5 g of yeast extract (Difco), and 10 g of NaCl; it was supplemented with glucose (0.1%) for conjugation experiments and with $CaCl_2$ (2 mM) for use with P1. The minimal medium used was half-strength medium 56 (56/2) described by Monod et al. (14) supplemented with 0.2% glucose as carbon source and with vitamin and amino acid requirements. H-1 buffer-salts solution (11) was used for diluting and resuspending. Streptomycin sulfate (Drug Houses of Australia Ltd., Adelaide, South Australia) and nalidixic acid (a gift of Winthrop Laboratories, Ermington, New South Wales) were used at 200 μg/ml and 6 μg/ml, respectively.

Conjugation experiments. Conditions were as described by Adelberg and Burns (2), with the exception that lysogenic males were washed and resuspended in fresh broth to reduce the free phage concentration. Phage 186 antiserum (1 part in 150 of rabbit serum with $K = 550\ min^{-1}$) was added to neutralize the remaining free phage. If required, conjugation was interrupted by use of a vibratory device of the type described by Low and Wood (12), generously made available by P. Reeves. The male population was counterselected with streptomycin or, in the case of AB2528, phage T6 (8). Samples were plated on selective media for recombinants, or with indicator bacteria to assay for 186 prophage. To test isolated recombinants for 186 lysogeny, colonies were placed with a toothpick first onto plates seeded with 594, and then onto plates of 594 (186) as a control. When the female strain used was lysogenic for phage Mu1, the indicator bacteria used to score lysogeny were also lysogenic for Mu1 (AT2092).

P1 transductions. P1*vir* lysates were prepared on the donor strain by a confluent plate lysis technique. All lysates were recycled on the same host once before use. In all transductions except those involving *nalB*, the same lysate from 594 was used throughout.

TABLE 1. *E. coli K-12 strains utilized*[a]

Strain	Mating type[b]	Relevant genotype	Source
AB2528	Hfr	*ilvD leu thi str*^{r} (λ)	A. J. Pittard; same origin as AB313
KL16	Hfr	*thi str*^{s}	P. Reeves
KL98	Hfr	*str*^{s} (λ)	A. J. Pittard
KL164	Hfr	*thyA thi nalB*^{r}	CGSC 4304; B. Low
JP190	F$^-$	*tyrA ilvD argH metB thi str*^{r} *tsx*^{r}	A. J. Pittard
JP264	F$^-$	*his ilvD argH metB thi str*^{r} *tsx*^{r}	A. J. Pittard
AT713	F$^-$	*lysB argA cysC str*^{r}	A. J. Pittard
AT2092	F$^-$	*pheA purF thi his str*^{r} *tsx*^{r} (Mu1)	A. J. Pittard
AT2457		*glyA thi*	UTH 4133[c]
H-724		*his tyrA trp purC guaB thi*	UTH 4069[c]
H-888		*his trp purG thi*	UTH 4105[c]
PA3306		*nadB purI argH*	UTH 4664[c]
594	F$^-$	*galK galT str*^{r}	D. S. Hogness; H-111

[a] The genetic symbols are those used by Taylor (19).

[b] The origins of the Hfr strains are shown in Fig. 1. In addition, 186 or 186*p* lysogens of many of the above strains were prepared.

[c] Obtained from T. S. Matney.

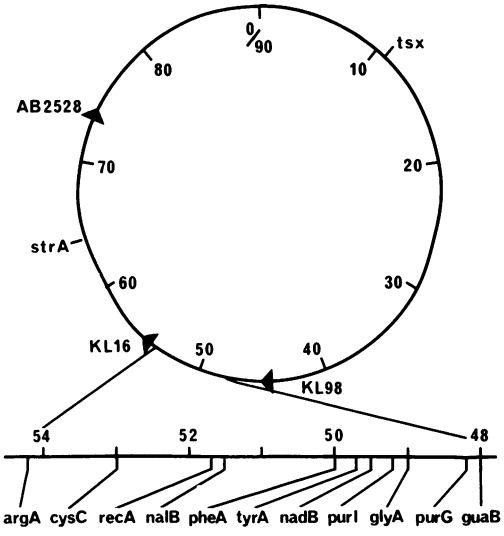

FIG. 1. *E. coli linkage map, based on that of Taylor (19), showing origins of transfer of Hfr strains and detail of the region 48 to 54 min.*

Lysates prepared on 186 lysogens were treated with 186 antiserum. For transduction, phage were added at a multiplicity of 0.05 to an exponential-phase culture of the recipient at 3×10^8 viable cells/ml, and the mixture was incubated for 30 min at 37 C. Then the cells were washed and resuspended in H-1 before being plated on selective medium. The three-factor transductions, some of which involved 186*p*, were performed at 30 C throughout.

Analysis of transductants. Transductants were purified through short streaks on selective medium before being tested for 186 lysogeny as above. A P1-resistant 186 indicator strain could not be isolated, necessitating further streaking away from P1 on selective medium before retesting many transductants. Where *nalB* was involved, transductants were patched out on selective medium, and the thickly grown patches were replicated to an L plate containing nalidixic acid. In this case, the patches were used in lieu of short streaks as preliminary purification before testing for 186 lysogeny.

RESULTS

Uninterrupted matings. Since Jacob and Wollman located *att*186 in the lower left quadrant of the *E. coli* linkage map (9), we commenced with the Hfr strain AB2528 (186). Recombinants for the *his* marker (39 min) were selected after 90 min of conjugation with JP264, and 55% (65 of 120) of these were found to be lysogenic for 186, compatible with Jacob and Wollman's assignment. Conjugations (Table 2) involving Hfr KL16 (186) showed linkage of (186) to *tyrA* (50 min), *purF* (44 min) and *pheA* (50 min), whereas Hfr KL98 (186) did not transfer (186) to *his*$^+$ or *purF*$^+$ recombinants of AT2092 in 60 min. Thus, *att*186 is located between the origins of KL16 and KL98, and close to *pheA*. The recovery of markers distal to (186) confirms the absence of zygotic induction with this prophage (Woods and Egan, *in preparation*).

Interrupted mating. The results of an interrupted-mating experiment between KL16 (186) and AT2092 are shown in Fig. 2. Prophage 186 enters close to and probably before *pheA*. The scatter of points for (186), and the lesser slope of its entry compared with *pheA*, reflect the efficiency of assay for the (186) marker. This method depends upon the appearance of a plaque on indicator bacteria due

TABLE 2. *Linkage of att186 to various genes in crosses of KL16 (186) × F⁻*[a]

F⁻ auxotroph	Marker	Fraction of recombinants lysogenic for 186
JP190	*tyrA*	313/350
AT2092	*purF*	153/200
AT2092	*pheA*	188/200

[a] Recombinants for the markers in column 2 were selected after 60-min conjugation between KL16 (186) and the nonlysogenic F⁻ shown in column 1.

to spontaneous phage production in a recombinant. In reconstruction experiments, only 20% of bacteria lysogenic for 186 plated with indicator bacteria scored as plaques, and these plaques were poorly defined. This 20% plating efficiency would explain the (186) slope being one-fifth of the *pheA* slope.

The entry time of the *his* marker varied from experiment to experiment, and was always delayed in the presence of 186 antiserum compared with literature values (20). However, (186) always entered with *pheA*.

Similar matings were performed with JP190, and in these (186) entered 0.8 to 1.2 min before *tyrA*.

Two-factor P1 transductions. These transductions can be represented as X⁺ donor and X⁻ (186) recipient. X⁺ transductants were selected, and linkage of X to prophage 186 was determined by checking these transductants for the absence of 186 lysogeny. In each instance, transduction to a nonlysogenic X⁻ recipient was performed, and the transduction frequency (number of transductants/plaque-forming unit) for the X marker was found to be the same, regardless of the lysogenic state of the recipient. For each recipient strain, transduction of markers unlinked to (186) invariably gave lysogenic transductants (200 of 200), so that the absence of prophage among transductants for some markers reflects linkage of the prophage to those markers. Markers checked in this manner were *trp* (25 min), *his* (39 min) *purF* (44 min), *guaB* (48 min) *purG* (48 min), *argA* (54 min), and *ilvA* (74 min).

For these two-factor transductions, P1*vir* propagated on 594 was used as donor, and the results recorded in Table 3 place *att*186 at about 51 min, between *pheA* and *cysC*.

Three-factor P1 transductions. Finer mapping within the *cysC-pheA* region was possible by use of the *nalB* marker, which we found to be about halfway between *cysC* and *pheA* (Table 4, lines 1 and 5). (Since an accurate locus for *nalB* has not been reported in the literature, we confirmed the order *argA-cysC-nalB* by three-factor P1 transduction.) Three-factor crosses were set up both to determine the relative order of *cysC*-(*nalB*, *att*186)-*pheA* and to seek evidence of disturbed linkage relationships indicative of actual integration of the prophage into the continuity of the bacterial chromosome. The results are recorded in Table 4.

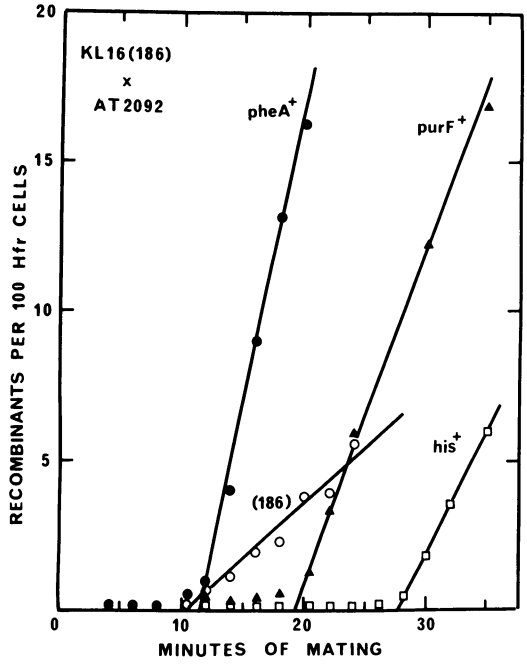

FIG. 2. *Marker entry curves for (186), pheA, purF, and his for an interrupted mating between KL16 (186) and AT2092.*

TABLE 3. *Two-factor P1 transductions from donor X⁺ to recipient X⁻ (186)*[a]

X	Nonlysogenic X⁺/total X⁺	Per cent co-transduction	Separation (min)
cysC	0/508	<0.2	
pheA	97/968	10.0	1.1
nadB	8/348	2.3	1.4
purI	5/392	1.3	1.5
glyA	0/542	<0.2	

[a] The markers X are shown in their order on the *E. coli* genetic map. The separation X from *att*186 is calculated according to Wu (24).

DISCUSSION

With uninterrupted matings, it was first established that *att*186 was between the origins of KL16 at 55 min and KL98 at 44 min. After

TABLE 4. *Three-factor transductions involving att186, nalB, and pheA or cysC*

	Donor[a,b] markers	Recipient[a,c] markers	Analysis of *phe*$^+$ or *cys*$^+$ transductants[d]				Cotransduction frequency
	A B	a b	AB	Ab	aB	ab	*nalB-pheA*
1.	*nal*^{r} 186$^-$ *phe*$^+$	*nal*^{s} 186$^-$ *phe*$^-$	29		499		29/528
2.	*nal*^{r} 186$^-$ *phe*$^+$	*nal*^{s} (186) *phe*$^-$	16	10	38	462	26/526
3.	*nal*^{r} (186*p*) *phe*$^+$	*nal*^{s} 186$^-$ *phe*$^-$	0	0	59	465	0/524
4.	*nal*^{r} (186*p*) *phe*$^+$	*nal*^{s} (186) *phe*$^-$	0	0	55	473	0/528
							cysC-nalB
5.	*cys*$^+$ *nal*^{r} 186$^-$	*cys*$^-$ nal^s 186$^-$	25		503		25/528
6.	*cys*$^+$ *nal*^{r} 186$^-$	*cys*$^-$ *nal*^{s} (186)	0	28	0	500	28/528
7.	*cys*$^+$ *nal*^{r} (186*p*)	*cys*$^-$ *nal*^{s} 186$^-$	0	29	0	495	29/524
8.	*cys*$^+$ *nal*^{r} (186*p*)	*cys*$^-$ *nal*^{s} (186)	0	26	0	521	26/547

[a] The prophage states are indicated as: 186$^-$, nonlysogenic for 186; (186), lysogenic for 186; and (186*p*), lysogenic for 186*p*.

[b] KL164 was used as donor.

[c] AT2092 and AT713 were used as recipients for *pheA* and *cysC* selections, respectively.

[d] Lines 1–4, *phe*$^+$; lines 5–8, *cys*$^+$.

interrupted matings, *att*186 could be placed close to *pheA*, and two-factor P1 transduction data put *att*186 at about 51.1 min, 1.1 min to the left of *pheA* on the *E. coli* K-12 map (19). This statement assumes that the presence of the prophage in the recipient chromosome but not in the donor chromosome does not seriously disturb transduction of that region, an assumption for which we can offer some justification later in the discussion.

The three-factor transductions performed concerned the relationship of *att*186 to *nalB*, a marker which has been placed in this region from interrupted mating (7). The results of these transductions (Table 4) show *nalB* to cotransduce at 5.5% with *pheA* and at 4.7% with *cysC*, frequencies which correspond to 1.2 min and 1.3 min, respectively. This *cysC-pheA* distance of 2.5 min compares favorably with 2.7 min reported by Willetts, Clark, and Low (22) from P1 transduction data, and with 3.0 min shown on the *E. coli* linkage map from conjugation data (20). We therefore place *nalB* equidistant from *cysC* and *pheA* at about 51.5 min.

With relation to *att*186, the important entries to note are numbers 4 and 8, in which both donor and recipient chromosomes carry the 186 prophage. When these are compared with transductions in the absence of prophage (entries 1 and 5, respectively) one notes that the *nalB-pheA* linkage drops from 5.5% to $<0.2\%$ because of the prophage, whereas the *cysC-nalB* linkage remains constant at 4.7%. Therefore, *att*186 lies between *nalB* and *pheA*, and the prophage is probably integrated into the continuity of the bacterial chromosome.

A further point to note from Table 4 is that, in transduction from the nonlysogenic donor to the lysogenic recipient (entry 2), the presence of the prophage in the recipient chromosome has marginal, if any, effect on the cotransduction frequency of *nalB* with *pheA* (a drop of 5.5 to 4.9%), and justifies our assumption of this fact earlier in this discussion. However, it should also be noted that the frequency of the recombinant class representing quadruple exchanges [*nal*^{r} (186) *phe*$^+$: 10 of 526] is not much less than that representing double exchanges (*nal*^{r} 186$^-$ *phe*$^+$: 16 of 526), suggesting that recombination is affected in this region.

The marker *recA* is mapped at 51.7 min (22), and an exciting prospect is the possibility of recovering a specialized transducing particle 186 d *recA*. The molecular weight of phage 186 deoxyribonucleic acid is 19.7×10^6 (21), and therefore its length is similar to the distance between *recA* and *att*186. Although it seems unlikely that a gene so far away could be recovered, the value of such a particle justifies our current attempts to isolate it.

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LITERATURE CITED

1. Abe, M., and J. Tomizawa. 1971. Chromosome replication in *Escherichia coli* K12 mutant affected in the process of DNA initiation. Genetics **69**:1–15.
2. Adelberg, E. A., and S. N. Burns. 1960. Genetic varia-

tion in the sex factor of *Escherichia coli*. J. Bacteriol. **79**:321-330.
3. Baldwin, R. L., P. Barrand, A. Fritsch, D. A. Goldthwait, and F. Jacob. 1966. Cohesive sites on the deoxyribonucleic acids from several temperate coliphages. J. Mol. Biol. **17**:343-357.
4. Calendar, R., and G. Lindahl. 1969. Attachment of prophage P2: gene order at different host chromosomal sites. Virology **39**:867-881.
5. Campbell, A. 1962. Episomes. Advan. Genet. **11**:101-145.
6. Gottesman, M. E., and R. A. Weisberg. 1971. Prophage insertion and excision, p. 113-138. *In* A. D. Hershey (ed.), The bacteriophage lambda. Cold Spring Harbor Laboratory, New York.
7. Hane, M. W., and T. H. Wood. 1969. *Escherichia coli* K-12 mutants resistant to nalidixic acid: genetic mapping and dominance studies. J. Bacteriol. **99**:238-241.
8. Hayes, W. 1957. The kinetics of the mating process in *Escherichia coli*. J. Gen. Microbiol. **16**:97-119.
9. Jacob, F., and E. L. Wollman. 1956. Sur les processus de conjugaison et de recombinaison chez *Escherichia coli*. I. L'induction par conjugaison ou induction zygotique. Ann. Inst. Pasteur (Paris) **91**:486-510.
10. Jacob, F., and E. L. Wollman. 1957. Genetic aspects of lysogeny, p. 468-500. *In* W. B. McElroy and H. B. Glass (ed.), The chemical basis of heredity. Johns Hopkins Press, Baltimore.
11. Kaiser, A. D., and D. S. Hogness. 1960. The transformation of *Escherichia coli* with deoxyribonucleic acid isolated from bacteriophage λ*dg*. J. Mol. Biol. **2**:392-415.
12. Low, B., and T. H. Wood. 1965. A quick and efficient method for interruption of bacterial conjugation. Genet. Res. **6**:300-303.
13. Matsushiro, A. 1963. Specialized transduction of tryptophan markers in *Escherichia coli* K12 by bacteriophage φ80. Virology **19**:475-482.
14. Monod, J., G. Cohen-Bazire, and M. Cohn. 1951. Sur la biosynthèse de la β-galactosidase (lactase) chez *Escherichia coli*. La spécificité de l'induction. Biochim. Biophys. Acta **7**:585-599.
15. Rothman, J. L. 1965. Transduction studies on the relation between prophage and host chromosome. J. Mol. Biol. **12**:892-912.
16. Signer, E. R. 1966. Interaction of prophages at the att_{80} site with the chromosome of *Escherichia coli*. J. Mol. Biol. **15**:243-255.
17. Signer, E. R. 1968. Lysogeny: the integration problem. Annu. Rev. Microbiol. **22**:451-488.
18. Six, E. 1966. Specificity of P2 for prophage site I on the chromosome of *Escherichia coli* strain C. Virology **29**: 106-125.
19. Taylor, A. L. 1970. Current linkage map of *Escherichia coli*. Bacteriol. Rev. **34**:155-175.
20. Taylor, A. L., and C. D. Trotter. 1967. Revised linkage map of *Escherichia coli*. Bacteriol. Rev. **31**:332-353.
21. Wang, J. C. 1967. Cyclisation of coliphage 186 DNA. J. Mol. Biol. **28**:403-411.
22. Willetts, N. S., A. J. Clark, and B. Low. 1969. Genetic location of certain mutations conferring recombination deficiency in *Escherichia coli*. J. Bacteriol. **97**:244-249.
23. Woods, W. H., and J. B. Egan. 1969. A study of nonultraviolet-inducible coliphages. Proc. Aust. Biochem. Soc. **2**:79.
24. Wu, T. T. 1966. A model for three-point analysis of random general transduction. Genetics **54**:405-410.