Chromosomal Relocation of Prophage-Associated Bacterial Genes¹

M. W. TAYLOR² AND C. YANOFSKY

Department of Biological Sciences, Stanford University, Stanford, California

Received for publication 3 December 1965

ABSTRACT

TAYLOR, M. W. (Stanford University, Stanford, Calif.), AND C. YANOFSKY. Chromosomal relocation of prophage-associated bacterial genes. *J. Bacteriol.* **91**:1469–1476. 1966.—Two distinguishable colony types, rough-edged and smooth-edged, were observed when tryptophan auxotrophs of *Escherichia coli* were transformed to tryptophan independence with DNA from the hybrid nondefective transducing phage $\lambda^{h\phi 80} T_1^S \text{ tryp } A^+B^+$, and with the helper phage λi^{434} . P1kc transduction experiments with cells of the two types of colonies as genetic donors showed that the $\lambda^{h\phi 80} T_1^S \text{ tryp } A^+B^+$ prophage was located at different regions of the *E. coli* chromosome. In cells of rough-edged colonies, the prophage was linked to the *tryp-cys* region, its normal location, whereas in cells of smooth-edged colonies the prophage was associated with the *gal* region. When transformation experiments were performed with a $T_1^R \text{ tryp}^-$ deletion mutant as recipient, and phage λi^{434} as helper, prophage localization was only detected at the *gal* region. Localization of $\lambda^{h\phi 80} T_1^S \text{ tryp } A^+B^+$ prophage near *gal* does not appear to be due to the formation of a recombinant phage carrying *tryp* A^+B^+ , but is due to some type of interaction between the genomes of $\lambda^{h\phi 80} T_1^S \text{ tryp } A^+B^+$ and the helper phage. When conditions comparable to those used in transformation studies were employed in transduction experiments, including the use of helper phage, two classes of transductants with either *cys* or *gal* linkage were also observed. To examine whether the location of the prophage on the *E. coli* chromosome had any effect on the ability of the prophage-associated *tryp* A^+ and *tryp* B^+ genes to function or respond to different repression conditions, specific activities of the A and B subunits of tryptophan synthetase specified by the phage genome were measured. Similar values were obtained regardless of the location of the prophage-associated *tryp* genes. Furthermore, the prophage-associated *tryp* genes, free from their normal operator region, permitted enzyme formation which was unaffected by repression or derepression conditions.

Kaiser and Hogness (6) demonstrated that deoxyribonucleic acid (DNA) from temperate coliphage λ carrying bacterial *gal*⁺ markers (λ dg) could be used to transform *gal*[−] cells of *Escherichia coli* to *gal*⁺. In an analogous manner, Taylor and Yanofsky (18) have shown that DNA from a λ - ϕ 80 hybrid phage carrying the wild-type tryptophan synthetase genes of *E. coli* ($\lambda^{h\phi 80} T_1^S \text{ tryp } A^+B^+$) could transform tryptophan auxotrophs to tryptophan independence. In the

latter studies, it was noticed that two distinguishable types of prototrophs were recovered—one type segregated tryptophan auxotrophs relatively frequently, whereas the other type did not. The explanation for this difference was sought, and the present paper reports the finding that the observed stability difference depends on the location of the prophage on the bacterial chromosome.

MATERIALS AND METHODS

A partial linkage map of *E. coli* showing the genetic region relevant to the present study is presented in Fig. 1.

Bacterial strains. The following strains were used: W1485, wild-type K-12; W1485 A23, a *tryp* A^- mutant (designated in this work A23[−]); W1485 $T_1^R \text{ tryp } A^-B^-7^{del}$, a $T_1^R \text{ tryp}^-$ deletion mutant lacking a chromosomal segment which includes the A and B genes of trypto-

¹ The material in this paper is taken in part from a dissertation presented to the Graduate School of Stanford University by Milton Taylor in partial fulfillment of the requirements for the Ph.D. degree.

² Predoctoral trainee of the U.S. Public Health Service. Present address: Department of Molecular and Cell Biology, University of California, Irvine.

FIG. 1. Partial linkage map of *Escherichia coli*, as given by Signer, Beckwith, and Brenner (12a): *gal K*, *gal T*, and *gal E* = structural genes for galactokinase, galactose 4-phosphate uridyl-transferase, and uridine di-phosphogalactose 4-epimerase, respectively; *att.* = attachment site of respective prophage; *bio* = biotin; *pyr* = pyrimidine; *pur* = purine; $T_1^{S/R}$ = phage T_1 sensitivity or resistance; *tryp A*, *B* = structural genes for tryptophan synthetase A and B proteins; *tryp C*, *D*, *E* = structural genes for indoleglycerol phosphate synthetase, phosphoribosyl anthranilate transferase, and anthranilate synthetase, respectively; *cys B* = cysteine B; ϕ = operator region.

phan synthetase and the T_1^S locus (this deletion will be designated *tryp A⁻B⁻-7^{del}*); and W1485 *his⁻ cys⁻ A23⁻* and W3101 *gal⁻ A23⁻*, nonlysogenic auxotrophs with the indicated markers. Various lysogenic and T_1 -resistant derivatives of these stocks were used.

Bacteriophage. Phage used were $i^h\phi^{80} T_1^S$ *tryp A⁺B⁺*, a nondefective high-frequency transducing (HFT) λ - ϕ^{80} hybrid carrying the bacterial tryptophan synthetase A and B genes, and T_1^S locus (18), kindly supplied by N. C. Franklin; λi^{434} , a λ -434 hybrid with the immunity of 434, kindly provided by A. D. Kaiser; and transducing phage P1kc (8).

Media. Tryptone broth agar contained 10 g of tryptone (Difco), 5 g of NaCl, 11 g of agar (Difco), and 1 liter of water.

Supplemented minimal medium was the minimal medium of Vogel and Bonner (18a), with 0.2% glucose and supplemented when desired with 0.2% acid-hydrolyzed casein, 20 μ g/ml of L-histidine, 30 μ g/ml of L-cysteine, 20 μ g/ml of L-tryptophan, and 1.5% agar.

Minimal galactose-agar: minimal medium (18a) with 0.4% galactose, 0.01% 2,3,5-triphenyl-2-H-tetrazolium chloride, 1.5% agar, and supplemented when desired with 20 μ g/ml of L-tryptophan.

Transformation. The transformation procedure employed has been described by Taylor and Yanofsky (18), and is a modification of the procedure developed by Radding and Kaiser (12). The DNA used in all experiments was prepared by the phenol extraction method (9) from purified preparations of the non-defective transducing phage $i^h\phi^{80} T_1^S$ *tryp A⁺B⁺*.

P1kc transduction. P1kc lysates were prepared by the confluent lysis technique (17). Transduction was performed at 37 C as described previously (20).

Enzyme assays. Procedures for the preparation of extracts and assaying of the A and B subunits of tryptophan synthetase, and anthranilate synthetase, have been described elsewhere (4, 13).

RESULTS

When a *tryp A⁻* mutant was used as recipient in transformation experiments with the DNA of phage $i^h\phi^{80} T_1^S$ *tryp A⁺B⁺* and λ or ϕ^{80} as helper phage, the transformed colonies invariably had rough edges. Single-colony isolates of such transformants, when grown overnight in broth, segregated tryptophan auxotrophs at high frequency—when an inoculum of approximately 10^7 cells per milliliter was employed, 20 to 30% of the cells

of a full-grown culture (ca. 2×10^9 bacteria per milliliter) were *tryp⁻*. When phage λi^{434} was used as helper under the same conditions, two distinct transformant colony types were observed, a rough-edged colony type as above (hereafter referred to as type RE colony) and a smooth-edged, larger colony type (hereafter referred to as type SE colony; Fig. 2). The cells of this second type of colony were considerably more stable than cells of type RE colony under comparable conditions—in a full-grown culture of cells from an SE colony there were usually 0.01 to 0.1% tryptophan auxotrophs.

The tryptophan biosynthetic genes of *E. coli* are known to be linked to a cysteine marker (20), and are cotransduced with this marker by phage P1kc. To investigate whether the difference in stability of the two types of colonies reflected a chromosomal location difference, P1kc lysates were prepared on both types of transformants. Lysates were also produced on W1485 and on the reference heterogenote A23/ $i^h\phi^{80} T_1^S$ *tryp A⁺B⁺* (prepared by lysogenizing A23 with transducing phage $i^h\phi^{80} T_1^S$ *tryp A⁺B⁺*). These P1kc lysates were used to transduce a *cys⁻ tryp⁻* double auxotroph to *cys⁺* or *tryp⁺* independence, or both (Table 1).

It can be seen that the linkage of *tryp* and *cys* in the reference heterogenote is considerably less than in the nonlysogenic parental strain W1485. This is not unexpected since the tryptophan A and B genes are likely to be separated from the bacterial *tryp* region by phage genes, and thus are further from the cysteine marker than in the non-heterogenotic strain. With transformant-type RE, a cotransduction frequency similar to that obtained with the reference heterogenote was observed. When transformant-type SE was used as donor, there was no *tryp-cys* cotransduction, suggesting either another chromosomal location for the prophage or its presence in an unattached form.

Transformation experiments with a deletion mutant recipient. Transformation experiments were also performed with a T_1^R *tryp⁻* deletion mutant, *tryp A⁻B⁻ - 7^{del}*, as recipient. These experiments were carried out under the same con-

FIG. 2. (A) Transductants obtained with the nondefective transducing phage $i^{\Delta h^{80}}T_1^S$ *tryp* A^+B^+ , and a *tryp* A^- mutant as recipient. (B) Transformants obtained with DNA from phage $i^{\Delta h^{80}}T_1^S$ *tryp* A^+B^+ , and λ^{434} as helper phage, and a *tryp* A^- mutant as recipient. Arrows point to SE colonies.

TABLE 1. Transduction with *Plkc* grown on various strains*

Donor	Recipient: <i>his⁻cys⁻tryp⁻(λ)(λi^{434})T₁^SR</i>		
	Transductants		
	<i>cys⁺ tryp⁻</i>	<i>cys⁺ tryp⁺</i>	Per cent <i>cys⁺ tryp⁺</i> joint transduction
W1485.....	551	343	61
<i>tryp⁺</i> transformant type RE.....	413	20	5
<i>tryp⁺</i> transformant type SE.....	ca. 1,000	0	<0.01
Reference hetero- note.....			
A23 ⁻ / <i>i^{Δh⁸⁰}</i> T ₁ ^S <i>tryp</i> A^+B^+	263	21	8

* *Cys⁺* transductants were selected on minimal agar supplemented with 20 μ g/ml of L-tryptophan and 30 μ g/ml of L-histidine. These transductants were replicated to minimal agar, and the *cys⁺ tryp⁺* colonies were scored. Independent estimates of cotransduction by plating on minimal agar with different supplements gave approximately the same values. In the most important case, with type SE as donor, joint transduction of *cys* and *tryp* was never observed.

ditions described above; i.e., the DNA was from phage $i^{\Delta h^{80}}T_1^S$ *tryp* A^+B^+ and phage λi^{434} was used as helper. Ninety *tryp⁺* transformants were isolated and purified, and each was tested for

lysogeny by spot testing on appropriate indicator bacteria. All 90 isolates were lysogenic for λi^{434} and $i^{\Delta h^{80}}T_1^S$ *tryp* A^+B^+ . In addition, they were all T_1^S , indicating that the T_1^S marker was introduced with the selected *tryp* A^+ and B^+ markers of the phage DNA. Ten of these transformants were selected for further study. All were found to segregate *tryp⁻* cells in the standard segregation test, but infrequently (10^{-3}), and thus in this respect were similar to type SE colonies. All 10 *tryp⁺* transformants were also found to yield lysates capable of transducing *tryp⁻* to *tryp⁺* at high frequency, indicating that they were heterogenotes carrying HFT phage. The results of *Plkc* transduction experiments with lysates prepared on these *tryp⁺* transformants are summarized in Table 2. *Cys-tryp* linkage was not observed with *Plkc* grown on any of the *tryp⁺* isolates, again suggesting the possibility of some other location for the prophage-linked *tryp* markers of type SE transformants. Since λi^{434} , the helper phage, has a prophage site in the vicinity of the galactose genes (5), the transformants were examined for *gal-tryp* linkage.

Linkage of *gal* and prophage-carried *tryp* markers. When a *tryp⁻ gal⁻* double mutant was used as a recipient in transduction experiments with *Plkc* grown on deletion mutant transformants (see Table 2), or on other transformants of type SE, linkage of *gal* and *tryp* was detected (Table 3). Cotransduction of *gal* and *tryp* is not observed

TABLE 2. Transduction with *P1kc* grown on transformed deletion mutants*

Donor	Recipient: <i>his⁻cys⁻tryp⁻ (λ)(λi⁴³⁴)T_{1,8}R</i>			
	Transductants			
	<i>cys⁺ tryp⁻</i>	<i>cys⁺ tryp⁺†</i>	<i>tryp⁺ cys⁻</i>	<i>tryp⁺ cys⁺</i>
<i>tryp A⁻B⁻-7^{del} (i^hh⁸⁰ T₁^S <i>tryp A⁺B⁺</i>) (λi⁴³⁴)</i>				
Isolate number				
1	148	0	500	0
2	137	0	582	0
3	184	0	773	0
4	161	0	204	0
5	49	0	500	0
6	73	0	327	0
7	93	0	368	0
11	143	0	300	0
22	163	0	500	0
Controls				
W1485	233	119	160	93
Reference heteroge- note				
A23 ⁻ /i ^h h ⁸⁰ T ₁ ^S <i>tryp</i> <i>A⁺B⁺</i>	407	51	98	16

* A *tryp⁻cys⁻* recipient was infected with *P1kc* grown on each of the donor strains indicated. Transductants were selected on minimal agar supplemented with 20 μg/ml of L-tryptophan, or 30 μg/ml of L-cysteine and replicated to minimal agar to score for cotransduction.

† Transductions mixtures were also plated at 10-fold higher levels in an attempt to detect *tryp-cys* cotransduction, but it was not observed with the test lysates.

when phage *P1kc* is grown on wild-type W1485, or on the reference heterogenote.

The finding of *gal-tryp* linkage with type SE transformants could be due to the production of a recombinant phage with the *tryp A⁺B⁺* genes and the prophage localization region of λi⁴³⁴. This recombinant presumably could be formed between i^hh⁸⁰ T₁^S *tryp A⁺B⁺* and λi⁴³⁴. Although recombinants with 434 immunity could be isolated from induced lysates of the transformants, none was found that could transduce *tryp⁻* auxotrophs to prototrophy. *Tryp⁺* transductants obtained with such lysates all carried i^hh⁸⁰.

Further evidence that a recombinant phage was not responsible for the *gal-tryp* linkage was obtained by transducing *tryp A⁻→tryp A⁺* with phage from ultraviolet-induced lysates of transformants of the type *tryp A⁻B⁻-7^{del} (i^hh⁸⁰ T₁^S *tryp A⁺B⁺*) (λi⁴³⁴)*. These transductants were then used as donors in *P1kc* transduction experi-

TABLE 3. *P1kc* transduction of a *gal⁻tryp⁻* auxotroph*

Donor	Recipient: <i>gal⁻tryp⁻ (λ)(λi⁴³⁴)T_{1,8}R</i>		
	Transductants		
	<i>gal⁺</i>	<i>tryp⁺</i>	<i>gal⁺ tryp⁺</i>
W1485	813	943	0
Reference heterogenote			
A23 ⁻ /i ^h h ⁸⁰ T ₁ ^S <i>tryp A⁺B⁺</i>	449	217	0
Transformant type SE—A23 ⁻ (λi ⁴³⁴) (i ^h h ⁸⁰ T ₁ ^S <i>tryp A⁺B⁺</i>)	1,619	1,092	225
<i>tryp A⁻B⁻-7^{del} (i^hh⁸⁰ T₁^S <i>tryp A⁺B⁺</i>) (λi⁴³⁴) #2</i>	500	460	80
<i>tryp A⁻B⁻-7^{del} (i^hh⁸⁰ T₁^S <i>tryp A⁺B⁺</i>) (λi⁴³⁴) #5</i>	279	219	28

* A *gal⁻tryp⁻* recipient was infected with *P1kc* grown on the donor indicated. Transductants were selected on minimal + galactose + L-tryptophan agar (*gal⁺* selected); minimal + glucose agar (*tryp⁺* selected), and on minimal + galactose agar (*gal⁺tryp⁺* selected).

ments to determine the location of the prophage-linked *tryp⁺* markers.

Table 4 shows that these transductants have the normal *cys-tryp* linkage observed with the reference i^hh⁸⁰ *tryp⁺* heterogenote. All isolates on induction produced HFT lysates, and were therefore heterogenotes. If a recombinant phage had been responsible for the *gal-tryp* linkage, it would have been expected to re-attach at the *gal* region, and *gal-tryp* linkage would have been retained. However, these "derived" transductants did not show *gal-tryp* linkage.

Double infection with λi⁴³⁴ and i^hh⁸⁰ T₁^S *tryp A⁺B⁺*. When a *tryp A⁻* mutant was infected simultaneously with both phage λi⁴³⁴ and i^hh⁸⁰ T₁^S *tryp A⁺B⁺* under conditions normally used for transduction with φ80 (direct infection of 10⁸ log-phase cells on minimal agar plates), only type RE transductants were recovered. Similar results were found if *tryp A⁻* (λi⁴³⁴) cells were infected with i^hh⁸⁰ T₁^S *tryp A⁺B⁺*. However, if conditions comparable to those used in the transformation assay were employed [cells preinfected with λi⁴³⁴ in 0.01 M tris(hydroxymethyl)aminomethane (pH 7.8), 0.01 M CaCl₂, 0.01 M MgSO₄], two classes of transductants were observed (SE and RE), although morphological differences were not so clearly defined as with transformants. The results of an examination of eight transductants is shown in Table 5. Since *gal-tryp*-linked transductants are recovered under such conditions, it would appear that i^hh⁸⁰ prophage localization

TABLE 4. *Plkc* transduction with lysates prepared on "derived" transductants

Donor	Recipients: <i>his⁻cys⁻tryp⁻(λ)(λi⁴³⁴)T_{1,8}^R</i> and <i>gal⁻tryp⁻(λ)(λi⁴³⁴)T_{1,8}^R</i>					
	Transductants					
	<i>cys⁺</i>	<i>cys⁺-tryp⁺</i>	Per cent joint transduction	<i>gal⁺</i>	<i>gal⁺-tryp⁺</i>	Per cent joint transduction
<i>tryp A⁻B⁻-7^{del}</i> transformant #2 (parental)	1,660	0	0	955	76	8
A23 ⁺ isolate 1.....	1,340	139	10.4	200	0	0
A23 ⁺ isolate 2.....	2,720	285	10.5	763	0	0
<i>tryp A⁻B⁻-7^{del}</i> transformant #5 (parental)	1,250	0	0	704	58	8.2
A23 ⁺ isolate 1.....	5,000	372	7.4	224	0	0
A23 ⁺ isolate 2.....	2,750	143	5.2	137	0	0
A23 ⁺ isolate 3.....	2,130	177	8.3	90	0	0
A23 ⁺ isolate 4.....	3,160	428	13.5	529	0	0

* Ultraviolet-induced lysates of transformants were used to transduce A23⁻ → A23⁺. *Plkc* was grown on some of the A23⁺ isolates and the *Plkc* lysates obtained were used in transduction experiments with *his⁻cys⁻tryp⁻(λ)(λi⁴³⁴)T_{1,8}^R* and *gal⁻tryp⁻(λ)(λi⁴³⁴)T_{1,8}^R* recipients.

TABLE 5. *Plkc* transduction of transductants obtained by double infection*

Donor	Recipients: <i>his⁻cys⁻tryp⁻(λ)(λi⁴³⁴)T_{1,8}^R</i> and <i>gal⁻tryp⁻(λ)(λi⁴³⁴)T_{1,8}^R</i>					
	Transductants					
	<i>cys⁺</i>	<i>cys⁺-tryp⁺</i>	Per cent joint transduction	<i>tryp⁺</i>	<i>gal⁺-tryp⁺</i>	Per cent joint transduction
<i>Tryp⁺</i> transductant						
Isolate number						
R-1	2,500	127	5	—	—	—
R-2	2,910	0	0	555	11	2
R-3	2,810	20	0.7	496	0	0
R-4	1,540	165	10.7	—	—	—
R-5	4,040	215	5.3	—	—	—
R-6	660	0	0	468	11	2.4
R-7	1,000	0	0	515	7	1.4
R-10	4,070	510	12.0	—	0	0
Controls						
W1485	1,410	600	42.5	516	0	0
Reference heterogenote						
A23 ⁻ / <i>i^hh⁴⁸⁰T_{1,8}^{tryp} A⁺B⁺</i>	6,750	681	10.1	192	0	0

* The recipients were infected with *Plkc* grown on isolates R1–R10, and transductants were selected on appropriately supplemented media. All isolates were doubly lysogenic for *λi⁴³⁴*, and *i^hh⁴⁸⁰T_{1,8}^{tryp} A⁺B⁺*.

near *gal* is not a feature of transformation per se but of some type of interaction between the DNA of the helper phage and the superinfecting phage *i^hh⁴⁸⁰ T_{1,8}^{tryp} A⁺B⁺*.

Regulation studies. Recent studies by Matsuhiro and co-workers (10, 11) and by Somerville and Yanofsky (14, 15) indicate that the tryptophan gene-cluster constitutes an operon, with the operator region in the vicinity of the anthranilate synthetase end of the operon. Ito and Crawford (4a) have shown that all the enzymes of the tryptophan biosynthetic pathway are co-

ordinately regulated. To examine whether the location of the heterogenetic fragment has any effect on its ability to function or to respond to different repression conditions, the specific activities of the A and B subunits of tryptophan synthetase were measured under conditions of repression (100 μg of L-tryptophan per ml) and depression. The level of anthranilate synthetase was also measured as an internal reference, since the anthranilate synthetase locus is not in the heterogenetic fragment. The results of the repression studies are presented in Table 6. Al-

TABLE 6 Specific activities of A and B components of tryptophan synthetase and anthranilate synthetase under conditions of repression

Genotype	Location of prophage-linked <i>tryp</i> A ⁺ B ⁺	Growth on 5 µg/ml of L-tryptophan (specific activities)			Growth on 100 µg/ml of L-tryptophan (specific activities)		
		A protein	B protein	Anthranilate synthetase	A protein	B protein	Anthranilate synthetase
W1485 wild type	No prophage	2.1	2.1	—	1.2	1.1	—
<i>tryp</i> A ⁻ B ⁻ -7 ^{del} (i ^h h ^{φ80} T ₁ ^S <i>tryp</i> A ⁺ B ⁺) [*]	Near <i>cys</i>	5.0	4.9	0.31	4.9	4.1	0.03
<i>tryp</i> A ⁻ B ⁻ -7 ^{del} transformant #2	Near <i>gal</i>	4.0	4.2	0.12	4.2	3.9	<0.01
<i>tryp</i> A ⁻ B ⁻ -7 ^{del} transformant #5	Near <i>gal</i>	5.5	4.7	0.66	4.7	3.3	0.035
<i>tryp</i> A ⁻ B ⁻ C ⁻ D ⁻ E ⁻ -8 ^{del} (i ^h h ^{φ80} T ₁ ^S <i>tryp</i> A ⁺ B ⁺) [†]	Near <i>cys</i>	6.7	5.7	—	5.7	5.8	—

^{*} *tryp* A⁻B⁻-7^{del} (i^hh^{φ80} T₁^S *tryp* A⁺B⁺)—*tryp* A⁻B⁻-7^{del} transduced with i^hh^{φ80} T₁^S *tryp* A⁺B⁺.

[†] *tryp* A⁻B⁻C⁻D⁻E⁻-8^{del}—deletion of *tryp* ABCDE transduced with i^hh^{φ80} T₁^S *tryp* A⁺B⁺. The phage is localized at a site linked to *cys*. Each culture grown for 16 hr in minimal medium supplemented with 5 or 100 µg/ml of L-tryptophan.

though A and B specific activities were relatively low in the heterogenotes, conditions which lead to repression of the A and B proteins of the nonlysogenic parent had no significant effect on the enzyme levels in either transductants or transformants, irrespective of the location of the prophage-associated *tryp* A⁺B⁺ genes on the bacterial chromosome.

In other experiments, derepression was attempted by transfer of cultures from a high-tryptophan medium to a tryptophan-free medium. Under these conditions, there was normally a 10-fold derepression of A and B protein activities in the wild-type control. No derepression of A and B protein activities was observed with any of the heterogenotes, regardless of the location of the prophage-linked *tryp* A⁺B⁺ genes.

DISCUSSION

The location of the tryptophan and galactose operons in relation to the λ, 434, and φ80 attachment sites on the *E. coli* chromosome are shown in Fig. 1. The hybrid nondefective transducing phage i^hh^{φ80} T₁^S *tryp* A⁺B⁺ lysogenizes normally near the tryptophan genes. However, as shown in the present study, when λi⁴³⁴ is used as helper in transformation experiments with DNA from i^hh^{φ80} T₁^S *tryp* A⁺B⁺, or when the hybrid phage is used as a superinfecting phage after λi⁴³⁴ infection, the tryptophan-transducing hybrid phage genome appears to be localized near the galactose operon. This conclusion is based on the demonstration, by use of P1kc transduction, that the hybrid phage genetic material is now linked to *gal*, and not to *cys* B. No *gal-tryp* linkage has ever been observed in single infection with phage i^hh^{φ80} T₁^S *tryp* A⁺B⁺.

The morphological difference noted in trans-

formant colonies when λi⁴³⁴ is used as helper is undoubtedly due to the stability of the transformants. Rough-edged (RE) colonies segregate sensitive cells at high frequency and produce free phage, as indicated by a large clear zone of lysis on a sensitive bacterial background. The cells at the edge of the colony are lysed to give the colony its rough-edged appearance. Unstable lysogeny of this type is typical of cells containing the non-defective transducing φ80 we have studied. The phenotype of the smooth-edged (SE) colony type, on the other hand, results from the greater stability and concomitant low rate of segregation of sensitive cells when the prophage i^hh^{φ80} T₁^S *tryp* A⁺B⁺ is located near the galactose genes. The explanation for the stability difference is not known although localization near *gal* always involves a second phage genome. The results presented in Table 4 indicate that localization of the hybrid phage near *gal* has not resulted from recombination yielding a phage carrying *tryp* A⁺B⁺ and the attachment site of λi⁴³⁴. If a recombinant phage had been formed, it seems unlikely that it would prefer to lysogenize near *tryp* when it initially was found near *gal*.

In contrast to the results with the A gene point-mutant recipient, all the examined transformants of the deletion mutant *tryp* A⁻B⁻ - 7^{del} showed *gal-tryp* linkage. In view of this finding it seems likely that the φ80 attachment site in this mutant has been altered in some manner. Although the deletion mutant *tryp* A⁻B⁻ - 7^{del} is lysogenized readily with φ80h⁻ (or i^hh^{φ80} T₁^S *tryp* A⁺B⁺ from a phenotypically mixed lysate), no *tryp* C or *tryp* D transducing phage were recovered in an induced LFT lysate of *tryp* A⁻B⁻ - 7^{del}(φ80h⁻). An examination of the *tryp*⁺ transductants resulting from infection of *tryp* A⁻B⁻ - 7^{del} at low mul-

tiplicity with $i^h\phi^{80}T_1^S$ *tryp* A^+B^+ from a phenotypically mixed lysate showed that localization of the prophage could occur at other sites on the bacterial chromosome. Similar results have been obtained in studies of lysogenization of deletion mutants with various $\phi 80$ stocks (N. C. Franklin, *personal communication*).

In the transformation system developed by Kaiser and Hogness (6) for phage λ and λ dg DNA, and by Taylor and Yanofsky (18) for $i^h\phi^{80}$ DNA, helper phage is essential for the recovery of infectivity. Dussoix and Arber (3) reported that restricted λ grown on *E. coli* C is unable to act as helper phage when *E. coli* K-12 is the recipient in transformation experiments, presumably due to the breakdown of phage DNA in the restricted host (2). However, *sus*⁻ mutants of λ are efficient helpers in *pm*⁻ hosts (Kaiser, *personal communication*), and λ helper phage can function in strains lysogenic for λ . These results would suggest that helper phage DNA must remain intact, but not necessarily be capable of replication, to function. Kaiser and Inman (7) and Strack and Kaiser (16) recently demonstrated that infectivity is decreased if the cohesive ends of the λ DNA are lost, either as a consequence of shearing or alteration by enzymatic action. These results, together with the finding reported here that λi^{434} mediates the location of $i^h\phi^{80}$, suggest that some type of physical interaction, possibly by joining of cohesive ends (19), takes place between the helper DNA and the infecting DNA. Preliminary studies (Taylor, *unpublished data*) indicate that λi^{434} , recovered from segregants of transformed cells which have lost $i^h\phi^{80}T_1^S$ *tryp* A^+B^+ , is altered both in ability to lysogenize and in density, implying some type of alteration as a consequence of the interaction.

The differences in P1kc cotransduction frequency of *gal* genes with *tryp* A in lysates prepared on transformants as donor, in comparison with superinfected transductants as donors (10% compared with 1%, Tables 3 and 5) may reflect the consequence of two different events. In superinfection transduction experiments, the primary event may be lysogenization by λi^{434} at the λ or 434 attachment site, followed by the insertion of $i^h\phi^{80}T_1^S$ *tryp* A^+B^+ within this prophage due to a greater affinity of the infecting phage genome for the prophage than for the bacterial chromosome. Calef et al. (1) have in fact recently reported that, in bacteria doubly lysogenic for λ , one phage genome appears to be inserted within the other. The different models of prophage interaction in double lysogenization of the type reported here are being examined.

Matsushiro et al. (11) reported that in bacteria heterogenetic for $\phi 80$ carrying various segments

of the tryptophan operon, but lacking anthranilate synthetase (*tryp* E), the expression of the heterogenetic fragment is nonrepressible by high concentrations of exogenous tryptophan. As shown in Table 6, similar results were found with the hybrid phage employed, irrespective of the position of the bacterial *tryp* genes on the chromosome. This implies that transcription and translation of these genes takes place equally well, regardless of location and in spite of the fact that they are situated far from the normal operator region. The low level of tryptophan synthetase A and B protein specific activity in these and similar heterogenotes (11) is not understood, but may reflect the control of the transcription of the *tryp* genes by phage genetic material.

ADDENDUM IN PROOF

Signer (J. Mol. Biol. 14:582, 1965) reported an analogous situation in which the *gal* genes carried by the defective transducing phage, λ dg, can be located near the *tryp* region, when $\lambda h\phi^{80}$ is used as helper phage in transduction experiments.

ACKNOWLEDGMENTS

This investigation was supported by grants from the National Science Foundation and the U.S. Public Health Service.

We express our thanks to J. Ito for aid with the enzyme assays, and to R. L. Somerville for an introduction to the peculiarities of phage $\phi 80$.

LITERATURE CITED

1. CALEF, E., C. MARCHELLI, AND F. GUERRINI. 1965. The formation of superinfection-double lysogens of phage λ in *Escherichia coli* K12. *Virology* 27:1-10.
2. DUSSOIX, D., AND W. ARBER. 1962. Host specificity of DNA produced by *Escherichia coli*. II. Control over acceptance of DNA from infecting λ . *J. Mol. Biol.* 5:37-49.
3. DUSSOIX, D., AND W. ARBER. 1965. Host specificity of DNA produced by *Escherichia coli*. IV. Host specificity of infectious DNA from λ . *J. Mol. Biol.* 11:238-248.
4. GIBSON, M. I., AND F. GIBSON. 1964. Preliminary studies on the isolation and metabolism of an intermediate in aromatic biosynthesis: chorismic acid. *Biochem. J.* 90:248-256.
- 4a. ITO, J., AND I. P. CRAWFORD. 1965. Regulation of the enzymes of the tryptophan pathway in *Escherichia coli*. *Genetics* 52:1303-1316.
5. KAISER, A. D., AND F. JACOB. 1957. Recombination between related temperate bacteriophages and the genetic control of immunity and prophage localization. *Virology* 4:509-521.
6. 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.
7. KAISER, A. D., AND R. B. INMAN. 1965. Cohesion

- and the biological activity of bacteriophage Lambda DNA. *J. Mol. Biol.* **13**:78-91.
8. LENNOX, E. S. 1955. Transduction of linked genetic characters of the host by bacteriophage P1. *Virology* **1**:190-206.
 9. MANDEL, J. D., AND A. D. HERSHEY. 1960. A fractionating column for analysis of nucleic acid. *Anal. Biochem.* **1**:66-77.
 10. MATSUSHIRO, A., S. KIDA, J. ITO, K. SATO, AND F. IMAMOTO. 1962. The regulatory mechanism of enzyme synthesis in the tryptophan biosynthetic pathway of *E. coli* K12. *Biochem. Biophys. Res. Commun.* **9**:204-208.
 11. MATSUSHIRO, A., K. SATO, J. ITO, S. KIDA, AND F. IMAMOTO. 1965. On the transcription of the tryptophan operon in *Escherichia coli*. I. The tryptophan operator. *J. Mol. Biol.* **11**: 54-63.
 12. RADDING, C. M., AND A. D. KAISER. 1963. Gene transfer by broken molecules of λ DNA. Activity of the left half molecule. *J. Mol. Biol.* **7**:225-233.
 - 12a. SIGNER, E. R., J. R. BECKWITH, AND S. BRENNER. 1965. Mapping of suppressor loci in *Escherichia coli*. *J. Mol. Biol.* **14**:153-166.
 13. SMITH, O. H., AND C. YANOFSKY. 1962. Enzymes involved in the biosynthesis of tryptophan, p. 794-806. In S. P. Colowick and N. O. Kaplan [ed.], *Methods in enzymology*, vol. 5. Academic Press, Inc., New York.
 14. SOMERVILLE, R., AND C. YANOFSKY. 1964. On the translation of the A gene region of tryptophan messenger RNA. *J. Mol. Biol.* **8**: 616-619.
 15. SOMERVILLE, R. L., AND C. YANOFSKY. 1965. Studies on the regulation of tryptophan biosynthesis in *Escherichia coli*. *J. Mol. Biol.* **11**: 747-759.
 16. STRACK, H. B., AND A. D. KAISER. 1965. On the structure of the ends of lambda DNA. *J. Mol. Biol.* **12**:36-49.
 17. SWANSTROM, M., AND M. H. ADAMS. 1951. Agar layer method for production of high titer phage stocks. *Proc. Soc. Exptl. Biol. Med.* **78**:372-375.
 18. TAYLOR, M., AND C. YANOFSKY. 1964. Transformation of bacterial markers and transfer of phage markers with DNA isolated from a λ - ϕ 80 hybrid carrying the tryptophan genes of *E. coli*. *Biochem. Biophys. Res. Commun.* **17**: 798-804.
 - 18a. VOGEL, H. S., AND D. M. BONNER. 1956. Acetylornithase of *Escherichia coli*: Partial purification and some properties. *J. Biol. Chem.* **218**: 97-106.
 19. YAMAGASHI, H., K. NAKAMURA, AND H. OZEKI. 1965. Cohesion occurring between DNA molecules of temperate phage ϕ 80 and Lambda or ϕ 81. *Biochem. Biophys. Res. Commun.* **20**: 727-733.
 20. YANOFSKY, C., AND E. S. LENNOX. 1959. Transduction and recombination study of linkage relationships among the genes controlling tryptophan synthesis in *Escherichia coli*. *Virology* **8**:425-447.