Chromosomal Relocation of Prophage-Associated Bacterial Genes

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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 \( \Phi hp^* T^8 \) tryp \( A^+B^+ \), and with the helper phage \( \lambda \). PIkc transduction experiments with cells of the two types of colonies as genetic donors showed that the \( \Phi hp^* T^8 \) 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 \( T^8 \) tryp\(^- \) deletion mutant as recipient, and phage \( \lambda \) as helper, prophage localization was only detected at the gal region. Localization of \( \Phi hp^* T^8 \) 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 \( \Phi hp^* T^8 \) 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 \( \lambda \) carrying bacterial gal\(^+ \) markers (\( \lambda dg \)) 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 \( \lambda dg \) hybrid phage carrying the wild-type tryptophan synthetase genes of E. coli (\( \Phi h480 T^8 \) 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\(^8 \) A\(^-\) B\(^-\) \( \lambda \), a \( T^8 \) tryp\(^- \) deletion mutant lacking a chromosomal segment which includes the A and B genes of trypto-
phan synthetase and the T₅ locus (this deletion will be designated tryp A⁻B⁻7del) and W1485 his⁺ cys⁻ A23⁻ and W3101 gal⁻ A23⁻, nonsylogenic auxotrophs with the indicated markers. Various lysogenic and T₁-resistant derivatives of these stocks were used.

**Bacteriophage.** Phage used were λh⁺T₅ tryp A⁺B⁺, a nondefective high-frequency transducing (HFT) λ-h₂₀ hybrid carrying the bacterial tryptophan synthetase A and B genes, and T₅ locus (18), kindly supplied by N. C. Franklin; λh⁺MK₁, 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 μg/ml of L-histidine, 30 μg/ml of L-lysine, 20 μg/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 μg/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 nondefective transducing phage λh⁺MK₁ T₅ 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 λh⁺MK₁ T₅ tryp A⁺B⁺ and λ or φ80 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 10⁶ cells per milliliter was employed, 20 to 30% of the cells of a full-grown culture (ca. 2 × 10⁸ bacteria per milliliter) were tryp⁺. When phage λh⁺MK₁ 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/λh⁺MK₁ T₅ tryp A⁺B⁺ (prepared by lysogenizing A23 with transducing phage λh⁺MK₁ T₅ 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 nonsylogenic 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 nonheterogenotoc 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 T₅ tryp⁻ deletion mutant, tryp A⁻B⁻ϕ₈₀, as recipient. These experiments were carried out under the same con-
Transductants obtained with the nondefective transducing phage $i^h800T_i^{B}$ tryp $A^+B^+$, and a tryp $A^-$ mutant as recipient. (B) Transforms obtained with DNA from phage $i^h800T_i^{B}$ tryp $A^+B^+$, and $\lambda^{54}$ as helper phage, and a tryp $A^-$ mutant as recipient. Arrows point to SE colonies.

Table 1. Transduction with Plkc grown on various strains

<table>
<thead>
<tr>
<th>Donor</th>
<th>Transductants</th>
<th>cys$^+$ tryp$^+$</th>
<th>cys$^+$ tryp$^-$</th>
<th>cys$^+$ tryp$^+$ joint transduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>W1485</td>
<td>551</td>
<td>343</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td>tryp$^+$ transformant type RE</td>
<td>413</td>
<td>20</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>tryp$^+$ transformant type SE</td>
<td>ca. 1,000</td>
<td>0</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Reference heterogeneote</td>
<td>A23$^+$/$i^h800T_i^{B}$ tryp $A^+B^+$</td>
<td>263</td>
<td>21</td>
<td>8</td>
</tr>
</tbody>
</table>

* Cys$^+$ transductants were selected on minimal agar supplemented with 20 $\mu$g/ml of L-tryptophan and 30 $\mu$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.

Conditions described above; i.e., the DNA was from phage $i^h800T_i^{B}$ tryp $A^+B^+$ and phage $\lambda^{54}$ was used as helper. Ninety tryp$^+$ transductants were isolated and purified, and each was tested for lysogeny by spot testing on appropriate indicator bacteria. All 90 isolates were lysogenic for $\lambda^{54}$ and $i^h800T_i^{B}$ tryp $A^+B^+$. In addition, they were all $T_i^{B}$, indicating that the $T_i^{B}$ marker was introduced with the selected tryp $A^+$ and $B^+$ markers of the phage DNA. Ten of these transductants 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$^+$ transductants 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$^+$ transductants 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 transductants. Since $\lambda^{54}$, the helper phage, has a prophage site in the vicinity of the galactose genes (5), the transductants 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 transductants (see Table 2), or on other transductants of type SE, linkage of gal and tryp was detected (Table 3). Cotransduction of gal and tryp is not observed.
when phage Plkc 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 λ^44. This recombinant presumably could be formed between Ph^440 T^8 tryp A+B+ and λ^44. 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 Ph^440.

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^- → tryp A^+ (λ^44). These transductants were then used as donors in Plkc transduction experiments to determine the location of the prophage-linked tryp^- markers.

Table 3 shows that these transductants have the normal cys-tryp linkage observed with the reference Ph^440 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 λ^44 and Ph^440 T^8 tryp A+B+ when a tryp^- mutant was infected simultaneously with both phage λ^44 and Ph^440 T^8 tryp A+B+ under conditions normally used for transduction with ph^80 (direct infection of 10^8 log-phase cells on minimal agar plates), only type RE transductants were recovered. Similar results were found if tryp A^- (λ^44) cells were infected with Ph^440 T^8 tryp A+B+. However, if conditions comparable to those used in the transformation assay were employed (cells preinfected with λ^44 in 0.01 M tris(hydroxymethyl)aminomethane (pH 7.8), 0.01 M CaCl^2, 0.01 M MgSO^4), 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 4. Since gal-tryp-linked transductants are recovered under such conditions, it would appear that Ph^440 prophage localization
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 λh-08OT18 tryp A+B+.

**Regulation studies.** Recent studies by Matsushiro 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 coordinately 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 4. Plkc transduction with lysates prepared on "derived" transductants**

<table>
<thead>
<tr>
<th>Donor</th>
<th>cys*</th>
<th>cys*-tryp*</th>
<th>Per cent joint transduction</th>
<th>gal*</th>
<th>gal*-tryp*</th>
<th>Per cent joint transduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>tryp A-B-7del transformant #2 (parental)</td>
<td>1,660</td>
<td>0</td>
<td>0</td>
<td>955</td>
<td>76</td>
<td>8</td>
</tr>
<tr>
<td>A23* isolate 1</td>
<td>1,340</td>
<td>139</td>
<td>10.4</td>
<td>200</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A23* isolate 2</td>
<td>2,720</td>
<td>285</td>
<td>10.5</td>
<td>763</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>tryp A-B-7del transformant #5 (parental)</td>
<td>1,250</td>
<td>0</td>
<td>0</td>
<td>704</td>
<td>58</td>
<td>8.2</td>
</tr>
<tr>
<td>A23* isolate 1</td>
<td>5,000</td>
<td>372</td>
<td>7.4</td>
<td>224</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A23* isolate 2</td>
<td>2,750</td>
<td>143</td>
<td>5.2</td>
<td>137</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A23* isolate 3</td>
<td>2,130</td>
<td>177</td>
<td>8.3</td>
<td>90</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A23* isolate 4</td>
<td>3,160</td>
<td>428</td>
<td>13.5</td>
<td>529</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* 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- (λi444)Ti, R and gal+ tryp- (λi444)Ti, A23 R recipients.

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

<table>
<thead>
<tr>
<th>Donor</th>
<th>cys*</th>
<th>cys*-tryp*</th>
<th>Per cent joint transduction</th>
<th>tryp*</th>
<th>gal*-tryp*</th>
<th>Per cent joint transduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryp+ transductant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isolate number</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-1</td>
<td>2,500</td>
<td>127</td>
<td>5</td>
<td>555</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>R-2</td>
<td>2,910</td>
<td>0</td>
<td>0</td>
<td>496</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>R-3</td>
<td>2,810</td>
<td>20</td>
<td>0.7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>R-4</td>
<td>1,540</td>
<td>165</td>
<td>10.3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>R-5</td>
<td>4,040</td>
<td>215</td>
<td>5.3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>R-6</td>
<td>660</td>
<td>0</td>
<td>0</td>
<td>468</td>
<td>11</td>
<td>2.4</td>
</tr>
<tr>
<td>R-7</td>
<td>1,000</td>
<td>0</td>
<td>0</td>
<td>515</td>
<td>7</td>
<td>1.4</td>
</tr>
<tr>
<td>R-10</td>
<td>4,070</td>
<td>510</td>
<td>12.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference heterogenote</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A23+/Phλ8T18 tryp A+B+</td>
<td>1,410</td>
<td>600</td>
<td>42.5</td>
<td>516</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>W1485</td>
<td>6,750</td>
<td>681</td>
<td>10.1</td>
<td>192</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* 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 λi444, and Phλ8T18 tryp A+B+. 
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 nonlyso-
genetic parent had no significant effect on the en-
zyme levels in either transductants or transformants, irrespective of the location of the pro-
phage-associated tryp A+B+ genes on the bacterial chromosome.

In other experiments, derepression was at-
ttempted 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 pro-
phage-linked tryp A+B+ genes.

**DISCUSSION**

The location of the tryptophan and galactose opers in relation to the λ, 434, and φ80 attach-
ment sites on the *E. coli* chromosome are shown in Fig. 1. The hybrid nondefective transducing phage λφ80 T8 tryp A+B+ lysoyzens normally near the tryptophan genes. However, as shown in the present study, when λ434 is used as helper in transformation experiments with DNA from λφ80 T8 tryp A+B+, or when the hybrid phage is used as a superinfecting phage after λ434 in-
fection, the tryptophan-transducing hybrid phage genome appears to be localized near the galac-
tose operon. This conclusion is based on the demon-
stration, by use of F1kc 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 λφ80 T8 tryp A+B+.

The morphological difference noted in trans-
formant colonies when λ434 is used as helper is undoubtedly due to the stability of the trans-
formants. 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 segmentation of sensitive cells when the prophage λφ80 T8 tryp A+B+ is located near the galactose genes. The explanation for the stability difference is not known although localization near gal always in-
volvea a second phage genome. The results pre-
sented in Table 4 indicate that localization of the hybrid phage near gal has not resulted from re-
combination yielding a phage carrying tryp A+B+ and the attachment site of λ434. 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− 7del 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− 7del is lysogenized readily with φ80h− (or λφ80 T8 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− 7del(φ80h−). An examination of the tryp+ transductants resulting from infection of tryp A−B− 7del at low mul-

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

| Genotype                      | Location of prophage-linked tryp A+B+ | Growth on 5 µg/ml of L-trypto-
|-------------------------------|--------------------------------------|phan (specific activities) | Growth on 100 µg/ml of L-trypto-
<table>
<thead>
<tr>
<th></th>
<th></th>
<th>A protein</th>
<th>B protein</th>
<th>Anthranilate synthetase</th>
<th>A protein</th>
<th>B protein</th>
<th>Anthranilate synthetase</th>
</tr>
</thead>
<tbody>
<tr>
<td>W1485 wild type</td>
<td>No prophage</td>
<td>2.1</td>
<td>2.1</td>
<td>—</td>
<td>1.2</td>
<td>1.1</td>
<td>—</td>
</tr>
<tr>
<td>tryp A−B− 7del (λφ80 T8 tryp A+B+)</td>
<td>Near cys</td>
<td>5.0</td>
<td>4.9</td>
<td>0.31</td>
<td>4.9</td>
<td>4.1</td>
<td>0.03</td>
</tr>
<tr>
<td>tryp A−B− 7del transformant #2</td>
<td>Near gal</td>
<td>4.0</td>
<td>4.2</td>
<td>0.12</td>
<td>4.2</td>
<td>3.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>tryp A−B− 7del transformant #5</td>
<td>Near gal</td>
<td>5.5</td>
<td>4.7</td>
<td>0.66</td>
<td>4.7</td>
<td>3.3</td>
<td>0.035</td>
</tr>
<tr>
<td>tryp A−B−C−D−E− 8del</td>
<td>Near cys</td>
<td>6.7</td>
<td>5.7</td>
<td>—</td>
<td>5.7</td>
<td>5.8</td>
<td>—</td>
</tr>
</tbody>
</table>

* tryp A−B− 7del (λφ80 T8 tryp A+B+)—tryp A−B− 7del transduced with λφ80 T8 tryp A+B+.
† tryp A−B−C−D−E− 8del—deletion of tryp ABCDE transduced with λφ80 T8 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.
timplicity with \( ^{i}p^h80 \) tryp \( A^{+}B^{+} \) from a phenotype 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 \( \lambda \) and \( \lambda dg \) DNA, and by Taylor and Yanofsky (18) for \( ^{i}p^h80 \) DNA, helper phage is essential for the recovery of infectivity. Dussoix and Arber (3) reported that restricted \( \lambda \) 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 \( \lambda \) are efficient helpers in \( pmc^{-} \) hosts (Kaiser, personal communication), and \( \lambda \) helper phage can function in strains lysogenic for \( \lambda \). 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 \( \lambda \) DNA are lost, either as a consequence of shearing or alteration by enzymatic action. These results, together with the finding reported here that \( \lambda \) mediates the location of \( ^{i}p^h80 \), suggest that some type of physical interaction, possibly by joining of cohesive ends (19), takes place between the helper DNA and the infecting phage.

Preliminary studies (Taylor, unpublished data) indicate that \( \lambda^i44 \), recovered from segregants of transformed cells which have lost \( ^{i}p^h80 \) \( T_{i}^B \) 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 Plkc cotransduction frequency of \( gal \) genes with tryp \( A \) in lyses 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 \( \lambda^i44 \) at the \( \lambda \) or 434 attachment site, followed by the insertion of \( p^h80 \) \( T_{i}^B \) 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 \( \lambda \), 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 heterogenous for \( \phi 80 \) carrying various segments of the trypophan operon, but lacking anthrani- lase synthetase (tryp E), the expression of the heterogenetic fragment is nonrepressible by high concentrations of exogenous trypophan. 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 trypophan 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, \( \lambda dg \), can be located near the \( tryp \) region, when \( ^{i}p^h80 \) is used as helper phage in transduction experiments.

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