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Isolation of Transducing Particles of φ80 Bacteriophage That Carry Different Regions of the *Escherichia coli* Genome

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ABSTRACT **It has been possible to mate two strains harboring F-prime (F′) factors and to isolate from such matings rare recombinants that behave as though the two episomes had fused. Thus, two genes not previously linked may be brought into close proximity.**

An F′ factor carrying the attachment site for φ80 was fused with one carrying the *met-ppc-arg* region of the chromosome. Lysogenization of such a strain, followed by induction, led to the isolation of φ80*arg*$^+$ and φ80*met*$^+$ transducing phages. This technique may be utilized as a general method for joining diverse bacterial genes to the genome of phage φ80.

Two autonomous F factors cannot normally replicate in the same *Escherichia coli* cell. Exceptional cases of apparent compatibility have been reported (1–5) but not, until recently, were they studied extensively enough to be understood. The use of recombination-deficient (Rec$^-$) (6) strains has facilitated investigation of such cases because integration of F factor into the chromosome is drastically reduced.

The present investigation was prompted by the isolation of strains carrying "fused" F′ factors. These were found during attempts to obtain mutants with impaired incompatibility functions (5). From zygotes carrying two different F′ factors, occasional progeny were obtained carrying a single F′ factor that contained the genomes of the two parental F's. Fusion of an F factor with a colicinogenic factor has already been described by Fredericq (7).

The possibility of fusing two F′ factors suggested this as a general method for linking genes from different regions of the chromosome. Moreover, it was reasoned that this technique could be used to eventually isolate portions of the bacterial genome. Thus, if an F′ carrying *att80*$^+$ (chromosomal attachment site for phage φ80) was fused with an F′ carrying a segment *x*, it should be possible, on subsequent infection, to isolate a φ80 particle carrying *x*. The results of our attempt to test this notion are described in this paper. The *trp-att80* and *met-ppc-arg* regions of the chromosome are normally separated by about 50 min of conjugation time (8). Two F′ factors, each carrying one of these segments, were successfully fused. From strains carrying such fused episomes, it was possible to obtain φ80 transducing particles carrying regions containing the *metB*$^+$ and *metF*$^+$ or the *argE*$^+$*C*$^+$*B*$^+$*H*$^+$ and *ppc*$^+$ genes. A φ80 phage carrying genes of the same region has been isolated by Konrad by a different method (personal communication).

Abbreviations: TMG, a medium containing Tris, Mg^{++}, NaCl, and gelatin (14); HFT, a lysate transducing a marker at high frequency.

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MATERIALS AND METHODS

Strain designations and symbols for genotypes follow the recommendations of Demerec *et al.* (9). The same 3-letter symbols are used for phenotypes, except that the first letter is capitalized and the symbols are not italicized.

Bacterial strains

F trp/7: F′ *trp*$^+$ *att80*$^+$*/thi trp argH thr leu recA str*
This strain carries a gene that confers resistance to φ80. The replication of the F′ factor is thermosensitive, occurring well at 30°C but not at 42°C. The episome has been derived from strain EC-8 (10) by the technique of Low (11); we selected for Trp$^+$ merodiploids in KL 250.

KLF 5/2463 (11): F′*metB*$^+$*F*$^+$ *ppc*$^+$ *argE*$^+$*C*$^+$*B*$^+$*H*$^+$*/pro argE thr leu his thi recA str*

KLF 15/JC 1553: F′ *his*$^+$*/leu his met arg recA str*

MA 3043: F′ *thr*$^+$ *leu*$^+$ *ara*$^+$*/thr leu pro his arg thi ara recA str*

KL 250: F$^-$ *thi argE trp thr leu pro his thy recA str*

KL EC-1: F$^-$ *thi met trp pro his thy str argEC-1* (deletion of *argEC*) (12)

EC-1 1359: F$^-$ *metB pro aroD argEC-1*

1012B: F$^-$ *trp thi.*

Phage strains

The φ80 used (λ 857 hφ80), isolated by E. Signer, contains a temperature-sensitive repressor, inactivated at 42°C. It was provided by Dr. G. Zubay.

Media

Neopeptone broth was prepared from a digest of beef heart to which was added Neopeptone (Difco) (10 g/liter) and NaCl (5 g/liter).

Z broth had the following ingredients per liter of distilled water: Bacto-tryptone (Difco), 10 g; yeast extract (Difco), 1 g; glucose, 1 g; NaCl, 8 g; 0.5 M $CaCl_2$ (added after autoclaving) 5 ml; Z plates contained 2% agar.

LBN broth (LB broth adjusted to neutrality) had the following ingredients per liter of distilled water: Bacto-tryptone, 10 g; yeast extract, 5 g; NaCl, 10 g; glucose, 1 g; and was adjusted to pH 7 with NaOH.

Soft neo top-agar was prepared from neopeptone broth by adding agar to a final concentration of 0.75%.

Minimal medium was medium A (13), supplemented with the required growth factors and 0.5% glucose as a carbon

source. Amino acids were added to a final concentration of 100 μg/ml and thiamine was added to a final concentration of 1.5 μg/ml. For solid media, Difco agar was added to a final concentration of 2%.

MacConkey arabinose plates were prepared from MacConkey Agar Base (Difco) by adding arabinose to a final concentration of 1%.

TMG suspension medium (pH 7.5) contained Tris, Mg^{++}, NaCl, and gelatin (14).

Matings

Unless otherwise specified, exponential-phase male cultures were mixed with exponential-phase female cultures in a ratio of 1:10 and were shaken slowly at 37°C for 1 hr. Appropriate dilutions were plated on selective media.

Preparation of lysates

A lysogenic strain was grown in minimal medium to mid-log phase. Four volumes of LBN broth were added, and the culture was grown with aeration in a side-arm flask at 33°C to a density of 3×10^8 cells/ml ($A_{580} = 0.15$), then incubated at 42–43°C for 10 min without agitation. After this temperature shock, the culture was aerated at 37°C for 4–5 hr, by which time lysis was usually complete. In some cases, good phage preparations were obtained from cultures not showing visible lysis. Chloroform was added; the culture was agitated slowly for 10 min at 37°C and then clarified by low-speed centrifugation. Titers were usually about 2×10^{10} phage/ml.

Phage titration

Strain 1012B was grown in neopeptone broth to mid-log phase. The culture was centrifuged and resuspended in 0.2 vol of Z broth. 0.1 ml of this concentrate was mixed with 0.1 ml of an appropriate phage dilution (in Z broth) and allowed to adsorb at 37°C for 10 min (15). After this incubation, 3 ml of soft neo agar at 45°C (to which a drop of 0.5 M $CaCl_2$ had been added) was added and the mixture was layered onto a Z plate. Plates were incubated overnight at 37°C and plaques were counted.

Lysogenization with ϕ80

0.1 ml of Z broth, containing 100 phage particles, was spread on a Z plate with about 2×10^8 late-log-phase bacteria. The plate was incubated for 2 days at 33°C. Bacteria were picked from the center of plaques, where turbidity was apparent, and were purified once on a minimal plate.

Transduction

The recipient was grown to late-log phase (about 2×10^9 cells/ml) in neopeptone broth, centrifuged, resuspended in 0.01 M $MgSO_4$, and starved in this medium for about 30 min with aeration at 37°C (16).

With a low-frequency transducing (LFT) lysate, the phage particles were concentrated before use in transduction to about 10^{11} phage/ml, and the bacteria were concentrated 10-fold in 0.01 M $MgSO_4$ to give 2×10^{10} cells/ml. Equal volumes of phage and bacteria were mixed, and allowed to adsorb for 10 min at 37°C. 0.2 ml of this mixture was spread on a selective plate, which was incubated at 33°C. Transductants appeared in 48 hr.

With a high-frequency transducing (HFT) lysate, a multiplicity of 5 was again used, but the mixture was diluted appropriately before plating. With fractions from density gradients, the fraction itself was diluted appropriately in Z (the mass of phage present in the fraction was estimated from its A_{260}) and 0.1 ml of this dilution was spread with 0.1 ml of a 0.01 M $MgSO_4$ suspension of bacteria containing 2×10^9 cells/ml (we used strain EC-1 1359) on a dry plate. Plates were incubated at 33°C.

Concentration of lysates and DNase treatment

Lysates were spun at 30,000 rpm in a Spinco model L ultracentrifuge in a no. 40 rotor for 1.5 hr at 3.5°C. Supernatants were decanted and discarded. Enough TMG medium was added to cover the pellets, which were suspended in this medium and allowed to stand at 4°C overnight. After this period, the pellets were evenly resuspended in TMG with a Pasteur pipette.

DNase suspension was added to a final concentration of 10 μg/ml, and the mixtures were incubated at room temperature for 30 min, after which they were spun for 10 min at 3000 rpm (14). The supernatants were used for CsCl centrifugation. In most cases, their titers were about 10^{11} plaque-forming units (PFU)/ml. (It appears from a limited number of experiments that DNase treatment is unnecessary.)

CsCl centrifugations

3 ml of sample was mixed with 2.3 g of optical grade CsCl (Harshaw Chemical, Cleveland), to give a final sample volume of about 3.5 ml. The starting refractive index was adjusted to approximately 1.3800. Samples were covered with mineral oil up to 2 mm from the top of the nitrocellulose tubes (14).

Suspensions were spun in a Spinco SW39 rotor at 24,000 rpm for 24 hr at 20°C. Tubes were then pierced at the bottom, and about 50 fractions of 0.07 ml each were collected. Fractions were dripped into tubes containing 1 ml of TMG, except for those on which refractive indexes were recorded, which were dripped into dry tubes; refractive indexes were read immediately. Gradients containing an initial titer of more than 10^{10} PFU/ml usually gave band(s) visible to the naked eye.

RESULTS AND DISCUSSION

Fusion of two F′ factors

In an attempt to isolate autonomous F factors with impaired incompatibility functions, a mating was performed between an F′ *his*$^+$/*his leu met arg recA* donor (KLF15/JC1553) and an F′ *thr*$^+$ *leu*$^+$ *ara*$^+$/*thr leu his ara recA* strain (MA3043) in F$^-$ phenocopy. Thr$^+$ Leu$^+$ His$^+$ progeny were selected. They occurred with a frequency of about 0.001% of the recipient parent cells; of these recombinants, about half could donate all F′ markers. Of the remainder most appeared to be *his*$^+$ revertants of the recipient strain. The strains that donated all the markers were further tested for possible linkage by mating with a *thr leu his recA* F$^-$ strain, selecting for inheritance of one of the F′ markers, and then testing the progeny for inheritance of the other F′ markers. There was 100% cotransfer of all F′ markers, which suggested that the two free F-factors had escaped the incompatibility barrier by becoming a single physical unit.

Since one of the F′ factors carried a wild-type gene for arabinose utilization, and the chromosome was *ara*, loss of the F′ *thr*$^+$ *leu*$^+$ *ara*$^+$ could be detected on MacConkey-arabinose medium. 16 spontaneous Ara$^-$ segregants were isolated and were all found to have lost both sets of F′ markers. This result supported the conclusion that the F′ factors had become integrated into a single physical unit.

Construction of (F′ *arg*$^+$ *met*$^+$ *trp*$^+$ *att80*$^+$)^{TS}/7

It seemed likely that the type of mating described above could be used to join chromosomal genes carried by one F′ to a phage-attachment site carried by another F′ factor. To this end, strain F trp/7 (F′ *trp*$^+$ *att80*$^+$/*thi trp argH thr leu recA*) was grown in minimal medium at 33°C and aerated for an additional 48 hr in the stationary phase, in order to convert it to the female phenocopy state. The culture was then mixed with an equal volume of an exponentially growing culture of KLF5/2463 (F′ *metB*$^+$*F*$^+$ *ppc*$^+$ *argE*$^+$*C*$^+$*B*$^+$*H*$^+$/*pro argE thr leu his thi recA*) and incubated at the same temperature for 60 min. Arg$^+$ His$^+$ Trp$^+$ Pro$^+$ recombinants were selected at 33°C and appeared in 2–4 days. They occurred at a frequency of about 0.0002%, significantly lower than for the His$^+$ Leu$^+$ strains referred to above (*Fusion of two F′ factors*). This can be explained by the fact that the F′ *trp*$^+$ *att80*$^+$ matings were performed at 33°C, instead of 37°C, because of the temperature sensitivity of this episome. Conjugation yields would be expected to be lower at the lower temperature (17).

Simultaneous transfer of *arg*$^+$ and *trp*$^+$ to strain KLEC-1 (F$^-$ *arg trp*) was observed with about 40% of the recombinants tested (55/136). Of these, 25% (14/55) could grow at 42°C in the absence of tryptophan and arginine. This may be understood if the thermoresistant F′ *arg*$^+$ *met*$^+$ and the thermosensitive *F′ trp*$^+$ have formed one replicon that, at least at the high temperature, relies on the controlling elements of the intact F′ *arg*$^+$ *met*$^+$ for replication. It is, however, also conceivable that the two episomes remain physically independent and that, at 42°C, F′ *arg*$^+$ *met*$^+$ assumes a helper function (18).

Many other strains gave ambiguous results when tested for temperature sensitivity. However, 16% (9/55) clearly required both arginine and tryptophan at 42°C, but not at 33°C, which suggests that *arg*$^+$ and *trp*$^+$ are part of the same genetic entity. In addition, it must be assumed that some or all of the genes that control replication in the thermoresistant F′ *arg*$^+$ *met*$^+$ factor have been impaired or lost. Despite our ignorance of the precise nature of these thermosensitive F′ *arg*$^+$ *trp*$^+$ entities, we used them for further experiments for two reasons: (*a*) the thermosensitivity of both phenotypes itself suggests physical connection of their genetic determinants; and (*b*) if the thermosensitivity occurred through a loop-out of the thermoresistant F factor, the distance between *att80* and either *met* or *arg* may have shortened, making the isolation of an *arg*$^+$ or *met*$^+$ transducing φ80 more likely.

TABLE 1. *Density gradients of φ80 arg*$^+$ *phage*

Phage no.	Lysis peak Fraction	Density (g cm^{-3})	Titer/ml	Transduction peak Fraction	Density (g cm^{-3})	Titer/ml
2	21	1.500	4 × 10^{10}	17	1.507	3 × 10^8
4A	22	1.500	7 × 10^9	19	1.505	7 × 10^7
5A	25	1.499	1 × 10^9	21	1.506	1 × 10^7
8	22	1.502	2 × 10^{10}	19	1.508	5 × 10^6
9	22	1.501	6 × 10^9	19	1.506	8 × 10^7
10	31	1.500	1 × 10^{10}	28	1.505	7 × 10^6
11	24	1.498	4 × 10^{10}	20	1.505	3 × 10^8
13	19	1.497	5 × 10^9	16	1.502	7 × 10^7
15	23	1.498	1 × 10^{10}	19	1.505	9 × 10^7
16	17	1.497	8 × 10^9	15	1.501	8 × 10^7
M15	20	1.498	6 × 10^{10}	16	1.504	8 × 10^7
Average		1.499				

Construction and lysogenization of (F′ *arg*$^+$ *met*$^+$ *trp*$^+$ *att80*$^+$)^{TS}/KL 250

The thermosensitive F′ *arg*$^+$ *trp*$^+$ episome was transferred to strain KL 250, since strain 7 is resistant to φ80. Exponential-phase neopeptone cultures of F *arg*$^+$ *trp*$^+$/7 and KL 250 were mated for 1 hr at 33°C, using phage T6 to interrupt the mating and to counterselect the donor. Appropriate dilutions were plated, selection being made for Arg$^+$ Trp$^+$ recombinants.

Three of the thermosensitive Trp$^+$ Arg$^+$ recombinants obtained from these matings were lysogenized with φ80. Since each lysogenized strain had two *att80* sites, one on the chromosome and one on the episome, the presence of φ80 on the episome had to be ascertained. Each of the (φ80)$^+$ F′ *arg*$^+$ *trp*$^+$/-KL 250 strains was mated with an F$^-$ *arg trp* (φ80)$^-$ strain (KL EC-1) and an isogenic (φ80)$^+$ derivative of this strain. Arg$^+$ Trp$^+$ recombinants were selected. Any donor carrying a φ80 prophage on the episome was expected to kill the (φ80)$^-$ recipient by zygotic induction and thus to give many fewer recombinants than with the (φ80)$^+$ female. Two of the three strains tested proved to have φ80 integrated at the episomal attachment site.

Transductions by lysates of F′ *arg*$^+$ *trp*$^+$ *met*$^+$ (φ80)$^+$/KL 250 strains

Lysates were prepared from the two strains containing φ80 on the episome by heat induction of the prophage. These were regarded as potential low-frequency transducing lysates for any gene close enough to the inserted φ80 prophage to be included in the rare transducing particles produced after induction. The lysates were, at any rate, expected to contain φ80 *trp*$^+$ particles, since *trp* is very close to *att80*. These particles were indeed found, at a frequency of 3 × 10^{-8}/PFU, thus providing evidence that transducing phage containing episomal markers was formed, since the chromosome was *trp*$^-$ To test for the presence of *met*$^+$ and *arg*$^+$ transducing particles, strain EC-1 1359, which carries a nonrevertible *metB* mutation and a polar *argEC* deletion (12), was used as recipient.

Met$^+$ recombinants were readily and repeatedly obtained, at an average frequency of 10^{-5}/PFU. (Since the chromosome of KL 250 carries *met*$^+$, as a control, a lysate of a lysogenic derivative of this strain *not* carrying any episome was used to transduce EC-1 1359. No recombinants appeared in this case.) On heat induction, more than 70% of these recombinants yielded *met*$^+$ HFT lysates (lysates transducing *met*$^+$ at high frequency). Among 288 *met*$^+$ transductants obtained using

TABLE 2. *Density gradients of φ80 met*$^+$ *phage*

Phage no.	Lysis peak Fraction	Density (g cm^{-3})	Titer/ml	Transduction peak Fraction	Density (g cm^{-3})	Titer/ml
A2	22	1.499	2 × 10^{10}	26	1.491	2 × 10^8
A4	24	1.501	2 × 10^{11}	30	1.492	3 × 10^8
13B A2 No. 6	26	1.507	9 × 10^{10}	30	1.501	3 × 10^8
6–24 A2 No. 5	12	1.498	9 × 10^{10}	14	1.495	1 × 10^9
Average		1.501				
A7	27	1.499	1 × 10^{10}	27	1.499	2 × 10^8

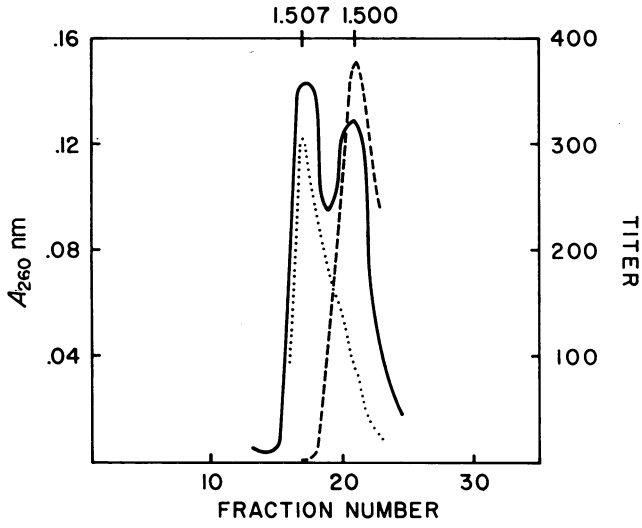

FIG. 1. CsCl density gradient analysis of lysate no. 2 (see Table 1), which contained a $\phi 80$ arg^+ phage. The *solid line* indicates A_{260}. The *dotted line* indicates transducing phage titers; numerical values indicated on the right-hand ordinate should be multiplied by 10^6/ml. The *dashed line* indicates infective phage titers; the units are $\times 10^8$/ml. The numbers at the top denote density.

these lysates, none had integrated ppc^+ or arg^+. However, of three lysates tested, all were found to transduce *metF* in addition to *metB*. These results are not surprising since *metF* and *metB* are 85–90% cotransducible by phage *363*, while *ppc* and *arg* are, respectively, 40 and 30% cotransducible with *metB* by this phage (19).

Arg^+ transductants were also found, but at a frequency about 100-times lower than for Met^+ transductants (10^{-10}/-PFU). Some of them, when induced, gave $argE^+C^+B^+H^+$ ppc^+ HFTs. (No joint transfer with *met* was observed.) The proportion of arg^+ HFT-producing transductants was quite variable from one experiment to another but was only estimated from rather low numbers of recombinants (at the most 20).

CsCl density gradient centrifugations of HFTs

Eleven of the arg^+ HFTs, and five of the met^+ HFTs, have been analyzed in a CsCl density gradient. A summary of the results is seen in Tables 1 and 2, and a typical gradient is plotted in Fig. 1. Recovery of active infectious phage from the gradients was always equal to at least 80% of the total input phage. The fraction numbers of the transduction and lysis peaks are included in the tables to give an idea of the number of fractions separating these peaks. The transducing titers of these HFT lysates, as seen from the tables, is 100- to 1000-times lower than the active phage titer, as was found by Gottesman and Beckwith (20). However, just as was observed by these investigators, the absorbance peaks of the transducing and plaque-forming particles are, in some lysates, of equal height (Fig. 1). In general, though, the proportion of transducing phage present is quite variable among lysates originating from different strains, and was as low as 5% of the total phage for one arg^+ HFT.

In all of the arg^+ HFT tested (Table 1), the transducing phage is denser than the wild type and is defective. For 4 of the 5 met^+ HFTs tested (Table 2), the transducing phage is less dense than the wild type and is defective. However, lysate A7 shows no significant difference in density between the peaks of plaque-forming and transducing ability. Further studies have shown that this lysate contains a nondefective transducing phage.

CONCLUSIONS

The existence of the $metB^+F^+$ and arg^+ppc^+ $\phi 80$ particles strongly supports the hypothesis that the F′ arg^+ met^+ and F′ trp^+ $att80^+$ have been fused and now replicate as one entity. This fusion presumably occurred by recombination between the F factors. These plasmids constitute a first, interesting application of a possibly general method for generating specific $\phi 80$ transducing particles, which have become an important tool in molecular biology. A choice between this method and the directed-transposition scheme devised by Gottesman and Beckwith (20) will mainly depend on the particular experimental conditions. The fusion of F′ factors could be the easiest way to isolate those chromosomal segments for which no marker related to sugar metabolism (thus detectable on colored indicator media) is available.

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