DRUG RESISTANCE OF ENTERIC BACTERIA

IV. Active Transducing Bacteriophage P1 CM Produced by the Combination of R Factor With Bacteriophage P1

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ABSTRACT

Kondo, Eiko (Gunma University, Maebashi, Japan), and Susumu Mitsuhashi. Drug resistance of enteric bacteria. IV. Active transducing phage P1 CM produced by the combination of R factor with phage P1. J. Bacteriol. 88:1266-1276. 1964.—During an investigation of the transduction of R factors with phage P1, a phage lysate capable of transducing the character of chloramphenicol resistance (CMr) in extremely high frequency was obtained. The transduction of the CMr character with the lysate was consistently accompanied by lysogenization with the phage used for transduction. This lysate exhibits no beneficial effect with normal P1, and no effect is produced by decreasing the multiplicity of infection. A single infection with the phage allows the formation of plaques as well as CMr lysogenic cells at the center of the plaque. Both the transducing and plaque-forming activities of the lysate were lost by neutralization with anti-P1 phage serum, and its absorption to the host bacteria was enhanced by the addition of Ca++. Thus, it was concluded that a derivative of P1 phage (P1 CM) was isolated which had not only the ability to transduce the CMr character but also the capacity to form plaques; i.e., the CMr gene of R factor is specifically associated with the genome of phage P1. No detectable differences were noted between P1 CM and normal P1 phage in density-gradient analyses in CsCl, in stability of lysogenization, in ability to transduce chromosomal markers, and in the mode of induction from lysogenic cells by ultraviolet irradiation. The instance of transduction of the CMr character described here may also be considered as an example of lysogenic conversion, in the sense that the alteration in CMr character is inseparable from lysogenicity.

The R factors which confer resistance to one or more of the drugs tetracycline (TC), chloramphenicol (CM), streptomycin (SM), and sulfanilamide (SA), and which can be transferred from one cell to another during conjugation among various strains of Enterobacteriaceae, are episomes (see Watanabe, 1963). They can be transduced with phage P1 (Nakaya, Nakamura, and Murata, 1960), P22 (Watanabe and Fukasawa, 1961), and ε (Harada et al., 1963). As indicated in a previous paper (Kondo, Harada, and Mitsuhashi, 1962), when the R factors which exhibit resistance to multidrugs are transduced with phage P1 in Escherichia coli K-12, their resistance markers are jointly transduced. However, in the case of the R factors carrying TC resistance, i.e., R14(TC.CM.SM.SA), R14(TC.SM.SA), and R4(TC.CM) (the numerical subscript designates the R factors, and drugs in parenthesis indicate the resistance conferred by the R factors), their resistance markers are transduced separately, and about 10 to 50% of the transductants accept resistance to TC alone. A majority of the R factors in transductants with P1 are transmissible by mixed cultivation, and these transductants are not lysogenized with P1, being sensitive to the phage.

In experiments on transduction with P1 and an R factor of a descendant derived from a CMr transductant, which is obtained by the separation of markers of R4(TC.CM) as described above, the CMr transducing phage which retained plaque-forming activity was isolated. The mode of transduction observed with this phage is different in various respects from that with P1 in transducing ordinary R factors or other genetic markers. These results strongly suggest that this phage is a new type of phage particle.

Transduction is a process of phage-mediated transfer of bacterial characters (Zinder and Lederberg, 1952). Based upon a series of studies made on λ transduction, possibilities of the defective nature of a transducing phage and the participation of a normal helper phage in the process were obvious (Arber, Kellenberger, and Weigle, 1957; Campbell, 1957). Phages P1 dl (Luria, Adams, and Ting, 1960) and φ80 dt (Matsushiro, 1963)
perform a similar type of transduction. With phages P1 and P22, transductions which occur in individual events proceed without lysogenization (Adams and Luria, 1958; Starlinger, 1958), and a fragment of bacterial genome, introduced by a phage, is integrated into the chromosome of a recipient cell. In such cases, transducing particles appear to be lacking entirely in phage genome. Accordingly, it may be concluded that the transducing particle is defective in phage function.

In lysogenic conversion (see Barksdale, 1959), for example, the somatic antigens O15 (Iseki and Sakai, 1953) and O34 (Harada, 1956) in Salmonella are converted by lysogenization [or immediately after infection (Uetake, Luria, and Burrous, 1958)] with ε15 and ε34, respectively. The phages possess a plaque-forming as well as a converting ability, and, therefore, a helper is unnecessary for the process. The origin of converting character is usually not known, whereas a transferred gene in transduction is obviously of bacterial origin.

Materials and Methods

Media. L broth (Lennox, 1955) was used as the routine liquid medium for phage propagation and transduction. L broth-agar (Lennox, 1955) was used as the bottom agar in phage propagation. The ordinary nutrient agar used as the bottom agar for plaque counts consisted of 1% beef extract, 1.5% peptone, 0.3% NaCl, and 1.5% agar (pH 7.2). The CaCl₂ solution was sterilized separately and was added to the bottom agar to a final concentration of 2 x 10⁻³ to 5 x 10⁻³ M. Soft agar (Lennox, 1955) was used as the top layer in the plating of phage. Heart infusion (HI) agar was used as the basal medium for preparing the selective plate used for transduction of drug resistance; it consisted of 1 liter of beef heart infusion, 10 g of peptone, 3 g of NaCl, and 20 g of agar; the pH was adjusted to 7.2. The heart infusion was prepared from beef heart; beef heart (500 g) was cut into small pieces and suspended in 1 liter of deionized water. After standing overnight in a refrigerator, the total suspension was heated at 100 C for 60 min. After cooling, a clear infusion was obtained by filtration. The complete and minimal eosin methylene blue (EMB) agar, a modification of Ledeburig's (1950), were used as the basal media for the selection of transductants of sugar fermentation and of nutritional markers, respectively. Bromothymol blue (BTB) lactose nutrient agar (ordinary nutrient agar with 1% lactose and 0.004% BTB) was used routinely for bacterial assay. Brain Heart Infusion (BHI, Difco) was used as the liquid medium for mixed cultivation. Tris(hydroxymethyl)-ammonomethane (tris) buffer (Luria et al., 1960) was used to prepare a purified suspension of phage particles, obtained by centrifugation, and a bacterial suspension for ultraviolet treatment. Saline (0.85% NaCl) was used for preparing dilutions of bacterial cells.

Drugs. A stock solution of chloramphenicol was prepared by dissolving 200 mg of the drug in a few milliliters of propylene glycol and making up to 100 ml with deionized water. Tetracycline hydrochloride was dissolved in the concentration of 2 mg/ml in deionized water before use. These drug solutions of the drugs were added to the melted agar to give a final concentration of 25 μg/ml. Dihydrostreptomycin sulfate was dissolved in deionized water to a concentration of 20 mg/ml, and an appropriate amount was added to the agar to give a final concentration of 200 μg/ml.

Microorganisms. The bacterial strains used are listed in Table 1. Shigella dysenteriae Sh/s was used as an indicator strain for phage P1 as well as its derivatives. E. coli K-12 F⁺ (prototroph) and W3110 were used as recipients for transduction of drug resistance. E. coli K-12 F⁺R⁺ was used as a donor of the R factor in the transduction. S. flexneri 3a was used as a recipient of the R factor in mixed cultivation.

P1ko (denoted as P1 in this paper) was used as the transducing phage.

The P1 CM phage was obtained by the following procedure. E. coli K-12 F⁺R⁺ (CM) and R⁺ (TC) were obtained by transducing E. coli K-12 F⁺ with phage P1 propagated on E. coli K-12 F⁺R₁₅ (TC, CM) (Kondo et al., 1962). The R (TC) and R₂₉ (CM) factors thus obtained were transferred to S. flexneri 3a by mixed cultivation from which S. flexneri 3a R₁₅ (CM) and R₂₉ (TC) were isolated. S. flexneri 3a, resistant to both TC and CM, was obtained by mixed cultivation of S. flexneri 3a R₁₅ (CM) and R₂₉ (TC). After three successive single-colony isolations, the resistance to TC and CM was still present. When resistance to TC and CM was transferred from S. flexneri 3a to E. coli K-12, upon mixed cultivation, most of the recipient cells of E. coli K-12 acquired resistance...
TABLE 1. Bacterial strains used*

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant characters</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Shigella dysenteriae Sh/s</em></td>
<td>F-, Lac-, SM*</td>
</tr>
<tr>
<td><em>S. flexneri 3a</em></td>
<td>F-, lac- (isolated from dysenteric patient)</td>
</tr>
<tr>
<td><em>Escherichia coli K-12</em> F+</td>
<td>F* (prototroph)</td>
</tr>
<tr>
<td><em>E. coli W1177</em></td>
<td>F-, T-, L-, B1-, lac-, mtl-, ara-, xyl-, mal-, gal-, SM*</td>
</tr>
<tr>
<td><em>E. coli W2984</em></td>
<td>F-, pro-*</td>
</tr>
<tr>
<td><em>E. coli W3110</em></td>
<td>F-, λ-</td>
</tr>
<tr>
<td><em>E. coli W4573</em></td>
<td>F-, lac-, ara-, mtl-, xyl-, mal-, gal-, SM*</td>
</tr>
<tr>
<td><em>E. coli 58-161 F</em></td>
<td>F-, M-</td>
</tr>
<tr>
<td><em>E. coli F</em>R<em>0(TC.CM)</em></td>
<td>F*, R14(TC.CM)</td>
</tr>
<tr>
<td><em>E. coli Tr 10-1,3</em></td>
<td>F*, R(TC.CM.SM.SA) [by transducing <em>E. coli K-12</em> F*+ with P1 propagated on <em>E. coli K-12</em> F<em>R</em>0(TC.CM.SM.SA)]</td>
</tr>
<tr>
<td><em>E. coli Tr 10-2</em></td>
<td>F*, R<em>0(CM) [by transducing <em>E. coli K-12</em> F</em>+ with P1 propagated on <em>E. coli K-12</em> F<em>R</em>0(TC.CM.SM.SA)]</td>
</tr>
<tr>
<td><em>E. coli Tr 10-7</em></td>
<td>F*, R<em>0(TC) [by transducing <em>E. coli K-12</em> F</em>+ with P1 propagated on <em>E. coli K-12</em> F<em>R</em>0(TC.CM.SM.SA)]</td>
</tr>
<tr>
<td><em>S. flexneri 3a R+(TC.CM)</em></td>
<td>F*, lac-, R(TC.CM) [derived by conjugation of <em>S. flexneri 3a R</em>0(TC.CM) and R*0(TC)]</td>
</tr>
<tr>
<td><em>E. coli KR 21</em></td>
<td>F*, R(TC.CM) [derived from <em>E. coli K-12</em> F*+ by conjugation with <em>S. flexneri 3a R</em>+(TC.CM)]</td>
</tr>
<tr>
<td><em>E. coli CT 6</em></td>
<td>F*, R*0(TC.CM) (by conjugation of Tr 10-2 and Tr 10-7)</td>
</tr>
<tr>
<td><em>E. coli Tr 30-5</em></td>
<td>F*, P1 CM* [by transducing <em>E. coli K-12</em> F*+ with P1 propagated on <em>E. coli K-12</em> F*+ (on P1)]</td>
</tr>
<tr>
<td><em>E. coli Tr 40-1 to -7</em></td>
<td>F*, P1 CM* [by transducing <em>E. coli K-12</em> F*+ with P1 CM propagated on <em>E. coli K-12</em> F*+ (on P1) propagated on <em>E. coli W3110</em>]</td>
</tr>
<tr>
<td><em>E. coli Tr 62-3</em></td>
<td>F*, λ-, P1 CM* [by transducing <em>E. coli W3110</em> with P1 CM propagated on <em>E. coli W3110</em>]</td>
</tr>
</tbody>
</table>

* R+ indicates cells which carry the R factor. P1 CM* indicates that the cell is lysogenic for P1 CM phage. Abbreviations: T, threonine; L, leucine; mtl, mannitol; xyl, xylose; ara, arabinose; mal, maltose; gal, galactose; B1, thiamine; M, methionine; pro, proline; lac, lactose.

to one or the other of the two drugs, and a few recipients acquired resistance to both drugs. One of the latter colonies, which was resistant to both TC and CM, was designated as *E. coli KR 21*. This strain had an immunity against phage P1, and the phage titer (2.3 × 10⁸) of lysate propagated on the strain was about 10³ times lower than that obtained by propagation of P1 on ordinary types of *E. coli K-12* R+ cells. The phage lysate propagated on *E. coli KR 21* was capable of transducing CM and TC resistance, but the transduction frequencies were 4 × 10⁻² and 1 × 10⁻⁴, respectively. The transduction frequency of CM resistance was 10⁴ to 10⁵ times higher than that of the ordinary R factor. To obtain a phage capable of transducing the CM* character at a high frequency, CM* transductants were acquired by transducing *E. coli K-12* F*+ with the lysate propagated on *E. coli KR 21*. One of these transductants was designated *E. coli Tr 30-5*. This strain released P1 CM phage.

To trace the origin of the P1 CM phage, transduction frequencies of lysates of strains which seem to have been involved in the derivation of the phage were measured. Of all the strains tested (*E. coli Tr 10-2, E. coli CT 6, E. coli Tr 16-1, 3, and *E. coli KR 21*), only *E. coli KR 21* produced lysates which transduced the CM* character with high frequency.

Methods for assay of phage and host organism. Assays of phage and host organism were carried out according to the method described by Adams (1950). An overnight L broth culture was used as the indicator. The rate constants for the adsorption of phage to bacteria and for the neutralization by antiphage serum were determined by the method of Adams (1950).

Preparation of phage lysates. The phage lysates, obtained by the agar-layer method (Swanson...
and Adams, 1951), were sterilized by shaking with chloroform. The chloroform was removed by aeration at 37 C before use. When the lysate was prepared from a single plaque, the plaque was stabbed with a sterile needle, and the needle was rinsed directly in soft agar warmed at 45 C. The soft-agar inoculum was plated together with the indicator cells to prepare the lysate.

**Transduction.** An overnight culture of the recipient bacteria, grown in L broth, was mixed with the phage in the presence of 2.5 x 10^{-3} M CaCl_2 to give about 2 x 10^8 cells per ml and a desired amount of phage lysate. After 20 min of incubation at 37 C, the unadsorbed phage and cells were separated, if necessary, by centrifugation, and the amount of unadsorbed phage was determined by the plaque method. Samples of the appropriately diluted adsorption mixture were plated on suitable selective plates. Colonies which developed on the selective plate after 48 hr of incubation at 37 C were counted. The transduction frequency was defined as the number of transductants (colonies developed on selective plate) per number of either adsorbed or input phage. As controls, sterility tests of the phage lysates and mutation tests of recipient bacteria (by plating without transducing phage) were conducted in most experiments.

**Transfer of R factors by mixed cultivation.** Equal volumes of the donor and recipient bacteria in their logarithmic phase of growth in BHI broth were mixed; each contained about 2 x 10^9 cells per ml. After incubation at 37 C for the desired length of time, suitable amounts of the mixture were spread on selective plates, which permitted the growth of only the recipient cells accepting drug resistance but not of parent cells.

**Ultraviolet treatment.** Cultures, grown overnight in L broth at 37 C, were centrifuged and resuspended to the same volume in tris buffer; 2 ml of the bacterial suspension were placed in a petri dish and agitated by hand during the irradiation. The source of ultraviolet light was a 15-w germicidal lamp (Toshiba Co., Tokyo, Japan) placed 40 cm from the object to be irradiated. After a desired time of irradiation, 2 ml of doubly concentrated L broth were added to each petri dish and incubated for phage multiplication. The viable-cell count of each sample was taken before the incubation.

**Lysogeny test.** The purified transductants, obtained by repeated cultivation in L broth containing anti-P1 phage serum or by single-colony isolation, were grown overnight at 37 C in L broth. A loopful of this culture was spotted on HI agar containing SM (200 µg/ml) and previously seeded with *S. dysenteriae* Sh/s (Bertani and Nice, 1954).

**Anti-P1 phage serum.** The P1 lysate obtained by propagation on *S. dysenteriae* was partially purified by centrifugation at 40,000 x g for 45 min, resuspended in tris buffer, and used as antigen. The antigen was injected intravenously into a rabbit daily (10^11 phage particles) for 5 days; 1 week after the last injection, the immune serum was obtained.

**RESULTS**

As described previously, in the transduction of R factors of *E. coli* K-12 with phage P1 (Kondo et al., 1962), no stable lysogenic clone had been found. However, *E. coli* Tr 30-5, one of the CM- transductants obtained with the phage lysate gained incidentally in the process described in Materials and Methods, was lysogenic. It released active phage particles into its broth culture, and the transduction frequency of the CM- character with its lysate was 10^{-1} to 10^{-2} (Table 2). *E. coli* Tr 30-5 possessed an immunity to phage P1 and could not produce the plaque of

<table>
<thead>
<tr>
<th>Phase of bacterial culture</th>
<th>No. of viable cells per ml</th>
<th>No. of phage particles per ml</th>
<th>Multiplicity of infection</th>
<th>Transduction frequency per adsorbed phage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stationary phase*</td>
<td>1.7 x 10^6</td>
<td>3.8 x 10^4</td>
<td>7.5 x 10^{-3}</td>
<td>1.2 x 10^{-2}</td>
</tr>
<tr>
<td>Logarithmic phase†</td>
<td>3.4 x 10^6</td>
<td>1.0 x 10^7</td>
<td>2.0 x 10^{-2}</td>
<td>1.2 x 10^{-1}</td>
</tr>
</tbody>
</table>

* Static culture in L broth after 18 hr.
† Overnight culture in L broth on standing was diluted 1:10 with L broth. A shake culture was used after 4.5 hr of incubation.
phage P1 by acting as a host, although it could adsorb P1 as well as the phage released from itself with almost the same efficiency as did the original E. coli K-12. The adsorption rate constant for the normal P1 was $3.5 \times 10^{-10}$ min$^{-1}$. The number of transductants obtained with the lysate obtained from E. coli Tr 30-5 was not increased by the addition of normal P1 phage. When the phage spontaneously released from E. coli Tr 30-5 was propagated on a CM-sensitive strain of E. coli, the lysate obtained exhibited a transduction frequency for CM$^r$ of about $10^{-1}$ or more.

To exclude the possibility that the CM$^r$ transduction is a result of the cooperation of two kinds of particles, i.e., defective particles that transduce the CM$^r$ character and active particles that form plaques, owing to the fact that E. coli Tr 30-5 is doubly lysogenic and releases the two kinds of particles, the following experiments were carried out.

As shown in Fig. 1, the linear relationship between the number of transductants and multiplicity of infection was found over a wide range from 4 to $4 \times 10^{-4}$, indicating the difference from the transduction of the gal$^+$ property with phage $\lambda$ (Campbell, 1957). All CM$^r$ transductants obtained at the multiplicity of infection of $4 \times 10^{-4}$ liberated the active plaque-forming phage with transduction frequencies similar to that of the lysate used in the initial infection (Table 3). From these results, it is concluded that the CM$^r$ character is transduced by a single particle which possesses a plaque-forming activity in addition to a transducing activity. This phage particle was designated as P1 CM.

**Properties of P1 CM.** P1 CM particles have the following characteristic properties of phage P1. (i) P1 CM particles are neutralized with anti-P1 phage serum. The neutralization rate constant of P1 CM and homologous P1 phage used to prepare the antisera was 147 and 168 min$^{-1}$, respectively. P1 CM lost completely both the transducing and plaque-forming abilities upon treatment with anti-P1 phage serum. (ii) It is well known that Ca$^{+2}$ ion is required for the adsorption of phage P1 to bacteria (Bertani, 1951). When the transduction with P1 CM was carried out in L broth without the addition of CaCl$_2$, the number of transductants diminished to about 40% of that obtained by the addition of CaCl$_2$. (iii) The host range of phage P1 CM is identical to that of phage P1. (iv) P1 CM gives uniformly turbid plaques which are scarcely dis-

**Table 3.** Plaque-forming titer and transduction frequency of the phage lysates released from CM$^r$ transductants obtained at a multiplicity of infection of $4 \times 10^{-4}$

<table>
<thead>
<tr>
<th>Strain</th>
<th>Plaque-forming titer per ml</th>
<th>Multiplicity of infection</th>
<th>Transduction frequency per input phage</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tr 40-1</td>
<td>$1.1 \times 10^9$</td>
<td>$2 \times 10^{-5}$</td>
<td>$5.2 \times 10^{-3}$</td>
</tr>
<tr>
<td>E. coli 40-2</td>
<td>$1.3 \times 10^8$</td>
<td>$3 \times 10^{-5}$</td>
<td>$4.8 \times 10^{-3}$</td>
</tr>
<tr>
<td>E. coli 40-3</td>
<td>$9.6 \times 10^7$</td>
<td>$2 \times 10^{-5}$</td>
<td>$1.0 \times 10^{-4}$</td>
</tr>
<tr>
<td>E. coli 40-4</td>
<td>$9.2 \times 10^7$</td>
<td>$2 \times 10^{-5}$</td>
<td>$4.1 \times 10^{-3}$</td>
</tr>
<tr>
<td>E. coli 40-5</td>
<td>$6.0 \times 10^7$</td>
<td>$2 \times 10^{-5}$</td>
<td>$1.2 \times 10^{-1}$</td>
</tr>
<tr>
<td>E. coli 40-6</td>
<td>$3.4 \times 10^6$</td>
<td>$2 \times 10^{-4}$</td>
<td>$1.3 \times 10^{-3}$</td>
</tr>
<tr>
<td>E. coli 40-7</td>
<td>$1.2 \times 10^6$</td>
<td>$2 \times 10^{-4}$</td>
<td>$4.2 \times 10^{-3}$</td>
</tr>
</tbody>
</table>

* Seven clones of CM$^r$ transductants were obtained at a multiplicity of infection of $4 \times 10^{-4}$ in the experiment shown in Fig. 1. Overnight cultures of the strains in L broth were centrifuged and the supernatant fluids were treated with chloroform and used as lysates.

![Graph](image-url)
tistinguishable from normal P1. When P1 CM is propagated lytically on host bacteria by the ordinary method, the titer of phage lysate obtained is usually $10^{10}$ to $4 \times 10^{10}$ per ml; in the case of normal P1, its titer is $4 \times 10^{10}$ to $8 \times 10^{10}$ per ml.

**Density-gradient centrifugation of P1 CM phage.** By the technique of density-gradient centrifugation in CsCl (Meselson, Stahl, and Vinograd, 1957), various defective transducing particles have been found to differ from the normal active phage particles in buoyant density (Weigle, Meselson, and Paigen, 1959; Ting, 1962; Shepard, 1962; Matsushiro, 1963). The P1 CM phage lysate, as well as the mixture of P1 CM and normal P1 phage, were subjected to density-gradient centrifugation in CsCl by use of a Spinco model E analytical ultracentrifuge, according to the method of Weigle et al. (1959). A phage lysate, propagated on *E. coli* K-12 F+ with phage spontaneously released from *E. coli* Tr 30-5, was added to a solution of CsCl buffered with tris buffer, pH 8.0 (Ting, 1962), and was centrifuged at 29,500 rev/min at 19.1 C. This lysate contained 75% P1 CM and 25% plaque-forming phage particles without the CM* transducing capacity (by plaque-center test described in a later section), and the total number of phage particles was $10^{10}$ per ml. A photograph of the ultraviolet-absorption pattern was taken upon attaining equilibrium at 12 hr. The pattern and microdensitometric trace of the photograph are shown in Fig. 2. A single adsorption band profile was observed, indicating that the transducing P1 CM phage and nontransducing phage in the lysate are indistinguishable in buoyant densities.

Furthermore, the mixture of normal P1 and P1 CM (lysate-C described in a later section) was subjected to preparative density-gradient centrifugation. A 3-ml sample of a mixture of about equal numbers of normal P1 and P1 CM was centrifuged by use of a swinging-bucket rotor. After centrifugation at 24,630 rev/min for 8.5 hr, the gradient was fractionated to 76 drops. After appropriate dilutions of each fraction, containing two drops, both transducing and plaque-forming activities were examined. The curves obtained by plotting transducing and plaque-forming activities against the number of drops are shown in Fig. 3. The peak of CM* transducing and plaque-forming activities appeared at almost the same position.

A difference in buoyant density between P1 CM and normal P1 phage was not observed. The plaque-forming particle, which lacked in transducibility of the CM* character and which was derived from P1 CM, was present in the P1 CM lysate, as will be described later. However, the particle was also indistinguishable from either normal P1 or P1 CM, as far as buoyant density was concerned.

**Ultraviolet induction of P1 CM.** Upon treatment with ultraviolet light, the production of P1 CM phage was induced. The optimal ultraviolet dose necessary was ascertained when the amount of cells surviving ultraviolet irradiation was 70 to 90%. After 3 hr of incubation, with shaking, the number of P1 CM phage particles of the supernatant fluid reached a maximal level. Representative results are shown in Table 4.

**Ability of P1 CM phage to transduce chromosomal genes.** An experiment on transduction of chromosomal markers with P1 CM lysate revealed that the lysate is capable of transducing lactose and xylose fermentation markers, as well as the nutritional markers, threonine, leucine, B<sub>1</sub>, proline, and methionine (Table 5). Most of
the transductants obtained were sensitive to CM and nonlysogenic with P1. CM-resistant transductants were also observed which were lysogenic with P1 CM. The lysogenization appears to be due to readsorption of the phage on the selective plate, since the P1 CM lysogenic transductants were found more frequently in the case of sugar fermentation markers, which were selected on a complete medium.

Transmissibility of CM resistance in P1 CM phage. The CM\(^\text{r}\) element in phage P1 CM does not appear to be transmissible by mixed cultivation, except through the infection of whole phage particle. Upon mixed cultivation with E. coli K-12 F\(^-\)P1 CM\(^+\), E. coli K-12 F\(^-\)/P1 (resistant to phage P1) does not accept a CM\(^\text{r}\) character. This problem is currently under investigation.

Heterogeneity of phage population in the lysate of P1 CM. As mentioned above, all CM\(^\text{r}\) transductants were lysogenic, and CM\(^\text{r}\) transductants without lysogenity were never found. Thus, an investigation was directed toward determining whether all lysogenized clones were resistant to CM. E. coli K-12 F\(^+\) was infected with a phage lysate prepared by propagation on E. coli K-12 F\(^+\) with phage released spontaneously from E. coli Tr 30-5, at a multiplicity of infection of 2.1. The correlation between lysogenicity and CM\(^\text{r}\) character of the surviving clones was then tested. Among the lysogenic clones, 93.4\% were found to be CM-resistant and 6.6\% were CM-sensitive. No clone was observed which was resistant to CM but nonlysogenic with P1.

The results show that almost all of the lysogenized cells are CM-resistant; however, lysogenized cells without CM resistance are also present. An active phage, lacking in the ability to transduce the CM\(^\text{r}\) character, was detected in the lysate which was propagated on E. coli K-12 F\(^+\) with phage particles spontaneously released from E. coli Tr 30-5.

A simple method for detecting the particle capable of transducing CM\(^\text{r}\) among those which form plaques was devised (plaque-center test). The existence of CM-resistant cells in the center of the plaque was determined by obtaining a sample from the center of the plaque with a needle and inoculating it on BTB lactose nutrient agar in the presence and in the absence of CM (25 \(\mu\text{g/ml}\)). A majority of the plaques contained CM-resistant cells at their center. The results were expressed as the percentage of

**TABLE 4. Induction of P1 CM phage from Escherichia coli Tr 62-3 by ultraviolet treatment**

<table>
<thead>
<tr>
<th>Ultraviolet irradiation</th>
<th>Cells surviving after irradiation (per ml)</th>
<th>No. of P1 CM phage particles per ml after incubation for</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2 hr</td>
</tr>
<tr>
<td>sec</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>1.5 \times 10^6 (100%)</td>
<td>1.6 \times 10^5</td>
</tr>
<tr>
<td>5</td>
<td>1.4 \times 10^6 (93%)</td>
<td>3.7 \times 10^4</td>
</tr>
<tr>
<td>10</td>
<td>1.1 \times 10^6 (73%)</td>
<td>4.7 \times 10^4</td>
</tr>
</tbody>
</table>

* After ultraviolet treatment according to the method described in the text, the bacteria were subjected to shaking culture at 37 C. At desired intervals, samples were withdrawn, and the number of P1 CM phage particles was determined.
plagues tested which contained CM-resistant cells, and this number was defined as the content of P1 CM phage among plaque-forming particles. The test gave a similar result when either E. coli K-12 or S. dysenteriae Sh/s was used as the host.

By the plaque-center test, P1 CM content of a phage lysate, spontaneously released from E. coli Tr 30-5, was about 92%, and that of a lysate prepared by propagation on E. coli K-12 F+ with the phage was about 79%. An attempt was made to locate a lysate composed of 100% P1 CM. About 40 plaques developing from a lysate, prepared by propagation on E. coli K-12 F+ with P1 CM phage of E. coli Tr 30-5, were isolated. A lysate was prepared from each of them after infection of the host E. coli W3110, and their P1 CM content was estimated by the plaque-center test. The average value of P1 CM content in these lysates thus obtained was 86%, and the highest value was 91%. The lysate, composed of 91% P1 CM, was denoted as the C-lysate.

One of the CMr transductants was obtained by infection of E. coli W3110 with C-lysate. This was denoted as E. coli Tr 62-3. When the plaque-center test was employed, 100% of the plaques formed by the phage particles, which were spontaneously released from E. coli Tr 62-3, contained CM-resistant cells. The phage titer of this lysate was as low as 106 per ml (Table 4). When some of the single plaques were propagated on E. coli W3110, the P1 CM content of the lysate thus obtained decreased to about 90% from the initial 100%. Upon losing the ability to transduce the CMr character of P1 CM, a phage particle appeared which retained the plaque-forming ability. This state was indicated also by the following experiment.

A single plaque, obtained by plating P1 CM released from E. coli Tr 62-3 on E. coli W3110, was picked with a sterile needle and suspended in two separate tubes containing soft agar. The tubes contained a suspension of ca. 104 and ca. 105 phage particles, respectively. The former phage suspension (ca. 105) was plated together with E. coli W3110 on an ordinary plate. The presence of cells resistant to CM in each plaque thus formed was tested by the plaque-center test described above; 94% of the plaques contained CM-resistant cells. The latter phage suspension (ca. 106) was plated with E. coli W3110 cells on an L broth-agar plate. After 18 hr of incubation at 37°C, the phage lysate was prepared, and the plaque-forming particles were counted. Upon propagation, the plaque-forming titer of the latter phage suspension (ca. 104 in total) increased to 1.2 × 106 (in total). An appropriate amount of phage lysate thus obtained was plated on E. coli W3110. After 18 hr of incubation at 37°C, the presence of CM-resistant cells in each plaque was investigated by the plaque-center test; 81% of the plaques thus formed contained CM-resistant cells.
**Discussion**

Phage P1 is capable of transducing not only chromosomal markers of bacteria (Lennox, 1955; Jacob, 1955) but also the epimorphs, such as F factor, λ prophage (Arber, 1960), or R factors (Nakaya et al., 1960; Kondo et al., 1962). The transductants with phage P1 in a single infection were generally sensitive to P1 (Adams and Luria, 1958). This indicates the absence or nondetectable existence of small portions of the P1 genome in the transducing particles, or the exclusion of P1 genome in the transductants. This fact also holds true in transduction of episomes with P1. The transduction frequency is increased by an appropriate ultraviolet irradiation of the phage or recipient cell, presumably owing to increasing the recombination frequency (Garen and Zinder, 1955), although that of the episomes, such as F factor, λ prophage (Arber, 1960), or R factor (Nakaya et al., 1961), is not increased. There is also a different type of transduction, in which a segment of the bacterial chromosome combines with some genetic components of the phage genome. In this case, there is a somewhat firm connection between a defective phage element and a piece of bacterial chromosome. These complex elements, such as λdg (Arber et al., 1957; Campbell, 1957), P1 dl (Luria et al., 1960) and φ80 dt (Matsushiro, 1963), can enter a mature phage particle to give rise to transducing particles which are defective in their phage functions.

In an analogy to these known facts, the functioning of the P1 CM phage particle, described in the present article, can be interpreted by postulating the existence of a complex genetic element, in which the P1 phage genome or its major portion is associated with the CM* determinant of R factor. However, the most striking difference noted is that this complex element, termed P1 CM*, still has the plaque-forming ability of phage P1.

The transduction efficiency of the CM* character with P1 CM was not affected by multiplicity of infection or by the addition of normal P1 phage. Therefore, the transduction of the CM* character with P1 CM is different from that with high frequency of transduction lysate, according to the terminology of Morse, Lederberg, and Lederberg (1956), which contains a comparable number of normal and transducing particles. The results shown in Fig. 1 may be interpreted in only two ways: that the plaque-forming particle has a transducing ability, or that two kinds of particles are present, one type being capable of forming plaques and the other type being capable of transducing. However, the latter possibility is excluded by the fact that all CM* transductants obtained at a multiplicity of infection of $4 \times 10^{-6}$ liberate the active plaque-forming phage with transduction frequencies similar to those of the lysate used in the initial infection. Moreover, according to the plaque-center test described above, most of the plaques which were developed by a single infection of phage particles contained cells resistant to CM in their center. Thus, it can be concluded that the transduction of a CM* character by the P1 CM phage does not require a helper phage, and that all of the essential phage functions are still retained by the P1 CM phage particles.

The acquisition of a CM* character by the recipient cell is always accompanied by the lysogenization of P1 CM, and neither the establishment of CM resistance alone, without lysogenization, nor the formation of a P1-defective CM-transducing particle was observed. Therefore, the CM*-conferring ability of P1 CM is entirely controlled by the phage, and the P1 element in P1 CM represents a nondefective genome of P1. The P1 CM can, therefore, be recognized as a "converting phage" rather than a "transducing phage," although the dividing line between these two expressions has become unclear, particularly since the discovery of λdg. The converting gene in a converting phage represents part of a converting phage genome, and the loss of a converting gene upon mutation is merely a rare event (Uetake and Uchida, 1959). In P1 CM, however, the association of a CM* gene with a P1 genome appears to be less firm than those of a typical converting phage, such as εN or εW. When the P1 CM is grown lytically on a host cell, the plaque-forming phage, lacking CM* transducing (or converting) ability, appears in a lysate at a very low percentage. However, the precise rate was not measured. The mechanism involved in losing the CM* character is not clearly understood but is now under investigation.

The P1 element in P1 CM represents a nondefective P1 genome, as far as we were able to determine. It possesses, at least, a genetic region which is responsible in conferring immunity
against phage P1, in stabilizing lysogeny, and in synthesizing tail-protein of P1, as well as in providing all information for replicating itself. The missing region in the P1 CM of a whole P1 genome was not analyzed at this time, because a mutant of phage P1 was not available. The buoyant density of P1 CM was scarcely different from that of the normal P1. If the amount of genetic material present in these two phages were accepted as equal, the formation of P1 CM from a nondefective P1 genome and CM resistance factor may be understood by postulating either (i) the presence of nonspecific genetic material in the P1 genome and an exchange of this material with the CM resistance factor, or (ii) the slightness of the CM portion that is included in the P1 phage coat. The CM portion in the P1 CM is presumably lacking in few markers of the original R factor, i.e., it does not exhibit both the transmissibility and the ability to inhibit F mating (Nakaya et al., 1960; Egawa and Hirota, 1962) which are observed in the original R factor (Kondo and Mitsuhashi, unpublished data). Such a loss of markers, other than the CM determinant, may support the supposition that the CM portion in the P1 CM is minute.

The origin of a converting gene in a converting phage is obscure, except for hypothesizing whether it originates from a bacteriophage itself or from bacterial genetic material. The fact that a converting phage, such as P1 CM, resulted from the association of a P1 genome with the CM determinant of a bacterial R factor and it exists stably, provides substantial evidence to support the hypothesis of Luria et al. (1958).

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Literature Cited


Luria, S. E., D. K. Fraser, J. N. Adams, and J. Burrous. 1958. Lysogenization, transduc-


