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Mechanism of pBR322 Transduction Mediated by Cytosine-Substituting T4 Bacteriophage

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Summary. A cytosine-substitution type mutant of bacteriophage T4 (T4dC phage) has been shown to mediate the transfer of plasmid pBR322. The transduction frequency was around 10^{-2} per singly infected cell at low multiplicity of infection. The transductants contained either a monomer or multimers of pBR322. The transducing capacity of T4dC phage was resistant to methylmethanesulfonate treatment. The results of Southern blotting experiments have indicated that the pBR322 DNA exists as head-to-tail concatemers in the transducing particles. The mechanism of transfer of pBR322 mediated by T4dC phages is discussed

Introduction

Bacteriophage T4 is one of the most intensively studied *Escherichia coli* phages and contains glucosylated hydroxymethylcytosine (glu-HMC) in its DNA in place of cytosine. Isolation of mutants termed T4dC, which incorporate cytosine instead of HMC, has made it possible to analyze the role of glu-HMC in the T4 genome (Snyder et al. 1976). T4dC phages have mutations in gene 42(dCMAP hydroxymethylase), gene 56(dCTPase), *den*B(endonuclease IV), and *alc* (=*unf*). When T4dC phages are grown in a restriction-deficient, non amber-suppressing host, the progeny phage particles contain cytosine-substituted DNA, which is susceptible to restriction endonucleases in vivo and in vitro (Velten et al. 1977; Wilson et al. 1977; Takahashi et al. 1978).

Recently, Wilson et al. (1979) reported that one of the T4dC phages, T4GT7, had a capacity to transduce plasmids as well as chromosomal markers. Little is known about the mechanism of T4GT-mediated transduction. Since the T4 genome is circularly permuted and encapsulated in a headful mode (Streisinger et al. 1964; Wood and Revel 1976), it would be quite interesting to know the physical state of the pBR322 genome in T4 phage particles.

In this paper we analyze the mechanism of T4dC phage-mediated transduction.

Materials and Methods

Bacterial Strains and Media. M9S medium (Takahashi et al. 1975) was used for growing bacterial strains. Titration of bacteria and phage was done on tryptone plates overlayed with 2.5 ml top-agar. Tryptone medium contained 1.0% Bactotryptone (Difco, Detroit, USA), 0.5% NaCl, 0.1% glucose, and 1.5% Bactoagar (Difco). *Escherichia coli* B40 (*sup*D) and B834(r^- m^- *sup*°) were described in a previous paper (Takahashi et al. 1978). B834 was used for propagation of cytosine-substituted T4 phages and as the recipient of transduction. Bacterial strains carrying pBR322 (Bolivar et al. 1977) were constructed by $CaCl_2$-treated transformation.

Cytosine-Substituted T4 Phages (T4dC) and Phage Preparation. T4dC(+) phage has point mutations in gene 42(*am*C87), gene 56(*am*E51), *den*B(*s*19), and *alc*(*unf*39) (Takahashi et al. 1978; Takahashi et al. 1979). T4dC(NB5060) phage has a deletion in the *r*II-*den*B-*ndd* region (NB5060) beside triple mutations of *am*C87, *am*E51 and *alc*(Wilson et al. 1979). T4dC phages were grown in B834 carrying pBR322 by the method described in previous papers (Takahashi et al. 1978; Takahashi et al. 1979). Phage particles were purified by differential centrifugations.

Cleavage of DNA. Cleavage of DNA was performed as described in a previous paper (Takahashi et al. 1979). Restriction endonucleases were purchased from Takara Shuzo, Co., Kyoto, Japan. Agarose gel electrophoresis was performed on a horizontal slab gel (0.7 cm × 14.5 cm × 13.5 cm) with 0.7% agarose (Seakem). The electrophoresis buffer consisted of 0.09 M Tris-0.09 M boric acid, pH 8.2, and 2.5 mM EDTA. Southern transfer (Southern 1975) and hybridization were done according to the method described in 'Manual for Genetic Engineering' (Davis et al. 1980). Hybridization was performed in a buffer consisting of 0.9 M NaCl, 50 mM Na_2HPO_4-NaH_2PO_4, pH 7.0, 5 mM EDTA, 0.3% SDS, 100 μg/ml denatured and sonicated calf thymus DNA, and ^{32}P-labeled pBR322 DNA at 38° C for 20 h. The probe DNA was prepared by labeling *Hae*III-cleaved pBR322 DNA with T4 DNA polymerase and α^{32}P-dATP according to the method of O'Farrell et al. (1980). Specific radioactivity of the probe was around 10^7 cpm per μg. Nitrocellulose filters of Schleicher and Schuell, Co. were used for DNA transfer.

Transduction by T4dC Phage. For transduction of pBR322, T4dC phages were grown in B834 carrying pBR322. Purified phages were diluted with M9 buffer (Takahashi 1978) and mixed with recipient cells grown in M9S medium. Usu-

with the same volume of T4dC phage suspension. After incubation at 37° C for 7 min for adsorption, the infected cells were centrifuged and washed once with M9 buffer. Portions of the infected cells were plated on tryptone agar plates containing 10 μg/ml ampicillin. Transduction frequencies are presented as Amp^r transductants per singly infected cell.

Isolation and Detection of Plasmid DNA. Rapid detection of plasmids from colonies was performed as follows: A medium sized colony was suspended in 10 μl TE buffer (10 mM Tris-HCl, pH 7.5 and 10 mM EDTA) containing 100 μg/ml egg-white lysozyme and incubated for 5 min on ice. To this were added 2 μl 2 N NaOH and 1 μl 10% SDS. The mixture was incubated at 37° C for 30 min. The solutions were neutralized with 2 μl 2N Tris-base and mixed with 3 μl 4 N NaCl. Samples were analyzed by agarose gel electrophoresis. For isolation of plasmid DNA on a preparative scale, the alkaline method mentioned above was scaled up. In this case, a cleared lysate was prepared by centrifuging the neutralized NaCl-containing sample at 30,000 rpm for 2 h. The cleared supernatant solution was treated with an equal volume of chloroform-phenol mixture (1:1). Nucleic acids were precipitated with ethanol and treated with RNAse and proteinase K. Finally plasmid DNA was separated by CsCl-EtBr centrifugation.

MMS Treatment of T4dC Phage. Methylmethanesulfonate (MMS, Eastmann, Co.) was added to 0.1 M Tris-HCl solution containing 10^9 plaque forming units (p.f.u.) of T4dC phage. After various times of 37° C incubation, aliquots were diluted with M9S medium containing 0.2 M $Na_2S_2O_3$ to inactivate MMS. The pBR322 transducing capacity was determined by infecting B834 cells at m.o.i. = 0.01 and plating on tryptone agar plates containing 10 μg/ml ampicillin.

Results

1. Transduction of pBR322 Mediated by T4dC Phages

To examine the transducing capacity of T4dC phages, two T4dC phages, T4dC(+) and T4dC(NB5060), were propagated in B834 carrying pBR322. When the lysates were used to infect B834, ampicillin resistant (Amp^r) colonies were obtained. Niether wild-type T4 grown in B834 carrying pBR322 nor T4dC phages grown in B834 without pBR322 gave rise to any Amp^r colonies. The transduction frequency by T4dC(NB5060) grown in B834 carrying pBR322 was around 2×10^{-2} Amp^r transductants per viable cell at a low multiplicity of infection (m.o.i.), which was 5-fold higher than that obtained with T4dC(+) phage. As shown in Fig. 1, the number of transductants increased with increasing m.o.i. in the range of 0.0001–0.5. At a higher m.o.i., the number of transductants decreased, presumably because of the killing effects of viable T4 phage particles. The linear relationship between the input phage particles and the number of transductants suggests that one transducing particle is capable of giving rise to one transductant.

2. Occurrence of Multimers in pBR322 Transductants

To analyze plasmids in T4dC-mediated transductants, Amp^r transductants were purified and the plasmid DNA

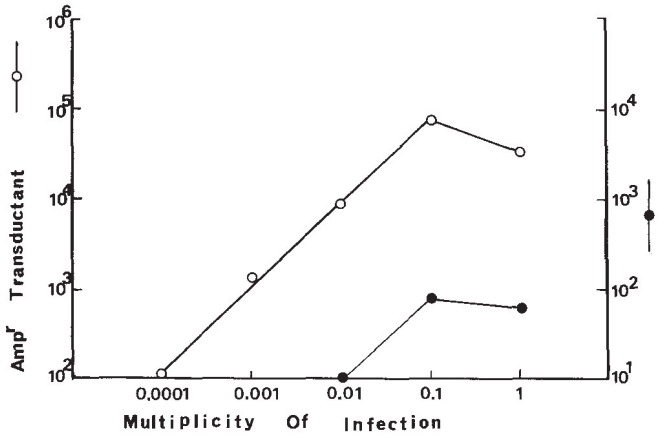

Fig. 1. Effect of multiplicity of infection on T4dC-mediated pBR322 transduction. T4dC(NB5060) phage propagated in either B834 carrying pBR322 (—○—) or B40 carrying pBR322 (—●—) was used as the donor phage. A culture of B834 was infected with T4dC phage at increasing m.o.i. ranging from 0.0001–1.0. The number of pBR322 transductants was determined by plating on ampicillin-containing plates

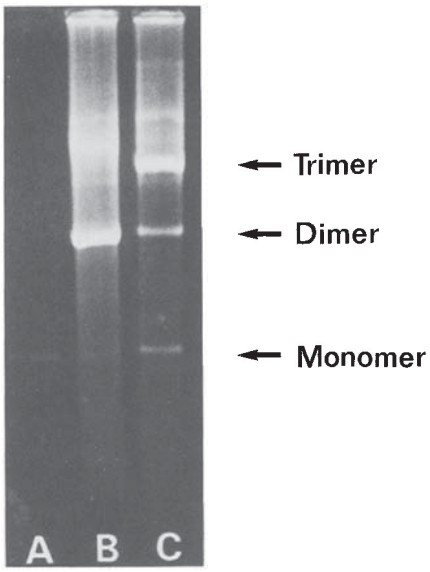

Fig. 2A–C. Agarose gel electrophoresis of plasmids from T4dC-mediated transductants. The DNA samples were electrophoresed through a 0.7% agarose gel. **A** Standard pBR322 DNA (monomer), **B** partially purified plasmid DNA from a transductant containing dimer plasmids, and **C** partially purified plasmid DNA from a transductant containing trimer plasmids

phoresis. Out of 40 Amp^r transductants examined, 23 clones were shown to contain the monomeric form of pBR322, and the remaining clones contained either dimers or trimers of pBR322. These multimers had a tendency to segregate to monomers of pBR322 (Fig. 2). After 20 generations in broth culture in the presence of ampicillin, more than half the multimers kept the original configuration. In contrast, when pBR322 was introduced by $CaCl_2$-transformation, all Amp^r transformants tested contained monomers. Therefore we suspected that the unusually high occurrence of multimeric forms in T4dC-mediated transductants might be due to multimeric forms of the pBR322 genome in the transduc-

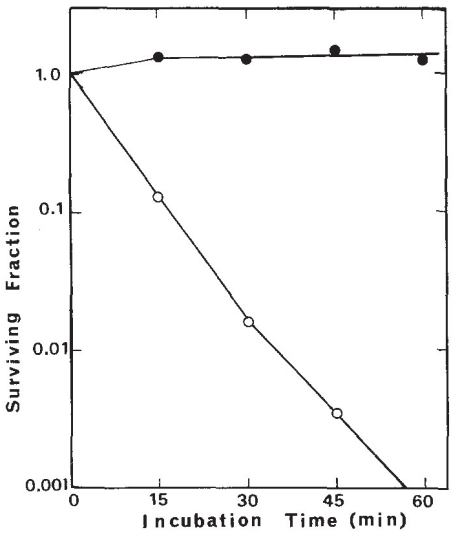

Fig. 3. Effect of MMS on viability and transducing capacity of T4dC phage. T4dC(NB5060) phage propagated in B834 carrying pBR322 was treated with 0.02 M MMS at 37° C. At times of incubation, the plaque-forming units (—o—) and transducing capacity (—●—) were determined

3. Methylmethanesulfonate (MMS) Resistance of the Transducing Particles

MMS inactivates phages by interacting with their DNA. To test the MMS sensitivity of T4dC transducing particles, T4dC(NB5060) phage propagated in B834 carrying pBR322 was treated with 0.02 M MMS at 37° C. Surviving fractions of plaque-forming units and the transducing capacity were determined. As shown in Fig. 3, the transducing capacity remained constant up to 120 min at which time the plaque-forming units were reduced by 10^{-5}. The transducing capacity was resistant also to ultraviolet irradiation (data not shown). These results suggest that the pBR322 DNA in the transducing particles is physically very stable.

4. Head-To-Tail Multimeric Form of pBR322 in the Transducing Particles

To analyze the physical structure of pBR322 molecules in the T4dC transducing particles, DNA from T4dC(NB5060) phage grown in B834 carrying pBR322 was examined by agarose gel electrophoresis. No visible DNA band corresponding to either CCC or linear forms of pBR322 was detected when the intact DNA was analyzed on the gel. However, when the DNA was cleaved with *Bam*HI endonuclease, a DNA band having identical mobility with that of the pBR322 linear form was observed (Fig. 4, lane B and D). Since T4 phage DNA has only one site for *Bam*HI (Takahashi et al. 1979; Wilson et al. 1980), the DNA band was conceivably derived from DNA of a larger size, presumably head-to-tail concatemers of pBR322 DNA.

To confirm the presence of multimeric forms of pBR322 molecules in the transducing particles, Southern hybridization experiments were performed. A ^{32}P-labeled pBR322 probe hybridized only with high molecular weight DNA when the intact DNA was used (Fig. 4, lane a). However, when the T4dC DNA was treated with restriction endonucleases *Bam*HI, *Pst*I or *Bal*I, which cleaves pBR322 DNA only once, then the pBR322 probe hybridized extensively with the band corresponding to the linear monomer of pBR322 DNA (Fig. 4, lanes b, e, f, and g). On the other hand, the probe hybridized only with high molecular weight DNA when the DNA was treated with *Kpn*I endonuclease which has no cleavage site in pBR322 (Fig. 4, lane c and d). These results indicates that the pBR322 DNA in the transducing particles exists as a head-to-tail concatemer and not integrated in the T4dC genome. Moreover, the intact size of DNA from the transducing particles had an identical size to that of DNA from the viable T4dC phage particles (Fig. 4, lane a). This result indicates that the transducing particles contain the same amount of DNA as the viable T4dC phage particles. Since the size of T4 phage DNA encapsulated in the phage particles is 166 kilobases (kb) (Wood and Revel 1976), the size of concatemieric DNA encapsulated in the transducing particles was estimated to be about 38 monomer equivalents of pBR322 DNA.

Discussion

T4dC phages grown in a suppressor-negative host carrying pBR322 were shown to transduce the plasmid at a high frequency. The frequency was around 10^{-2} per singly infected cell. Procedures for T4dC phage-mediated transduction are quite simple and fast. A number of pBR322 derivatives into which DNA fragments from various origins have

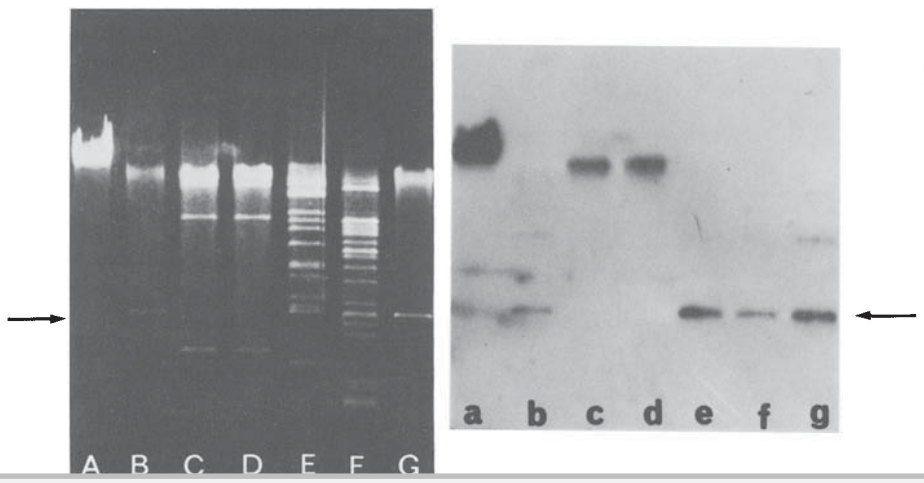

Fig. 4. Hybridization of ^{32}P-labeled pBR322 DNA to restriction fragments of DNA from T4dC(NB5060) phage grown in B834 carrying pBR322. The DNA fragments were separated by agarose gel electrophoresis and visualized by fluorescence with ethidium bromide (A–G). ^{32}P-labeled pBR322 DNA was hybridized to a Southern imprint of the fragments and hybrids were detected by autoradiography (a–g). A and a, intact DNA; B and b, *Bam*HI digest; C and c, *Kpn*I digest; D and d, *Kpn*I digest; E and e, *Bal*I digest; F and f, *Pst*I digest; G and g *Bam*HI digest. D (d) and G (g) samples were from the same DNA preparation. The arrow indicates the position of pBR322

been cloned, were also transduced at frequencies equivalent to that of pBR322 (Takahashi, Noguchi, and Saito, unpublished results). Since T4 phage particles can be stored for a long period without significant loss of transducing capacity, the T4dC-mediated transduction of plasmids can be used for preservation as well as transfer to plasmids into new hosts.

The T4 phage genome is circularly permuted and has a terminal redundancy. The phage DNA is encapsulated by a headful mode (Streisinger et al. 1964; Wood and Revel 1976). The results in this paper have shown that pBR322 DNA exists as head-to-tail linear multimers in the transducing particles. Moreover, the transducing particles were estimated to contain about 38 monomer equivalents of pBR322 DNA. DNA of pBR322 mostly exists in the CCC form in its host cells. Therefore, intramolecular recombinations are a prerequisite for producing linear multimers of pBR322 in the transducing particles and returning to CCC monomers in the recipient cells. The *rec*A function of the recipient cells is essential for this interconversion (Takahashi and Saito, manuscript in preparation).

T4dC phages grown in a suppressor-plus host carrying pBR322 were also able to transduce pBR322 although the transduction frequency was lower than that by T4dC phages grown in a suppressor-minus host carrying pBR322. DNA from T4dC phage grown in a suppressor-plus host carrying pBR322 was resistant to the restriction endonucleases *Eco*RI, *Sal*I, and *Pst*I (data not shown). Therefore, it is likely that both cytosine and HMC-containing pBR322 DNA in the transducing particles have been replicated by the T4 replication system, presumably in the mode of rolling circle (Streisinger et al. 1964; Wood and Revel 1976).

So far we have not succeeded in isolating transducing particles free of viable T4dC phage particles. However, the viable T4 particles can be selectively inactivated by MMS treatment or UV-irradiation. As viable T4 phage particles kill the cells infected with transducing particles, this selective inactivation of viable phage particles is convenient for T4dC-mediated transduction.

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