Parental to Progeny Molecular Recombination with Bacteriophage T7

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Parental deoxyribonucleic acid (DNA) molecules of bacteriophage T4 recombine with their progeny in a way which disperses pieces of the parental molecule over large numbers of the progeny DNA molecules. The parental contribution per progeny phage, or DNA molecule, which receives parental label, is about 5 to 7% of the phage DNA molecule (A. W. Kozinski, *Virology* 13:124, 1961). The fragment is inserted as a single piece with covalent repair at the intersection of the parental piece with the progeny polynucleotide chain (A. W. Kozinski and P. B. Kozinski, *Virology* 20:213, 1963; E. Shahn and A. W. Kozinski, *Virology* 30:455, 1966). Breakage and rejoining of DNA as a mode of recombination of bacteriophage has been proven so far only for T4 and λ (A. W. Kozinski, *Virology* 13:124, 1961; M. Meselson and W. O. Weigle, *Proc. Natl. Acad. Sci. U.S.* 47:857, 1961).

The purpose of this communication is to report that T7 behaves in a manner similar to T4; i.e., parental DNA becomes fragmented and inserted as a subunit covalently bonded to the adjacent progeny strand.

Analysis of the mode of parent-to-progeny transfer and recombination for bacteriophage T7 was conducted by use of analytical procedures and media similar to those described for T4 (A. W. Kozinski et al., *J. Virol.* 1:758, 1967). *Escherichia coli* B23 was grown to 3×10^8 bacteria/ml in heavy (5-bromodeoxyuridine-substituted) medium and was infected with ^{32}P -labeled (specific activity, 5.0 mc/mg) T7 bacteriophage at a multiplicity of infection of 3.0. Six minutes after infection the bacteria were sedimented to eliminate unadsorbed phage and resuspended in fresh heavy medium. (This was necessary as it was found that 20 to 50% of the parental phage were not adsorbed. The unadsorbed phage did not differ in density from adsorbed phage, though in a separate test they failed to adsorb to freshly added bacteria.) The bacteria were incubated until lysis (approximately 35 min), and the progeny phage were purified. The amount of parent-to-progeny transfer was estimated as follows: after washing the bacteria free of unadsorbed phage, a sample

of the infected bacteria was precipitated with trichloroacetic acid and considered as 100% recovery of the parental label; samples were taken for acid precipitation at each successive step of purification. Carrier phage was added after treatment of lysates with deoxyribonuclease and was plated at each step of purification thereafter; the ratio of ^{32}P to plaque-forming units (PFU) was calculated at each step of the procedure. Although there were substantial losses of material during purification (Table 1), the specific activity of ^{32}P /PFU remained essentially constant;

TABLE 1. Estimation of recovery of progeny phage during purification^a

Step in procedure	Recovery of acid-precipitable counts	Recovery of phages	^{32}P /PFU ratio
	%	%	
Washed bacteria.....	100		
Lysed bacteria.....	77.1		
After deoxyribonuclease treatment.....	58.3		
After filtration through Celite pad.....	26.1	100	0.261
High-speed centrifugation I ^b	6.17	28.7	0.214
Low-speed centrifugation ^c	6.76	31.0	0.218
High-speed centrifugation II ^b	2.41	11.9	0.203

^a After purification of progeny phage and estimation of the parent-to-progeny transfer, 100% recovery of acid-precipitable ^{32}P was measured (after the bacteria were washed free from unadsorbed parental phage); 100% recovery of phage was measured after filtration of the phages through a Celite pad. At this point, striking differences between T4 and T7 were noticed. The specific activity of ^{32}P /PFU was determined by dividing the percentage recovery of acid-precipitable counts per minute by the percentage recovery of phages at each subsequent step.

^b Centrifuged at 20,000 rev/min for 30 min in a Spinco 40 head.

^c Centrifuged at 6,000 rev/min for 10 min in a Sorvall GLC-1.

this shows that the losses were random and that the phage which were used in the following experiments were representative of the whole progeny population. The extent of transfer of parental material to the offspring and the observation concerning a large portion of unadsorbing phage in these experiments provide a confirmation of the original data of R. P. Mackal and L. M. Kozloff (*J. Biol. Chem.* **209**:83, 1954). After purification, the progeny phage were banded in CsCl density gradients and, after centrifugation, the gradient fractions were collected in vials. A sample from each vial was transferred and counted in a scintillation spectrometer. Another sample was plated for viable phage titer (Fig. 1). A small, conservative, light peak was usually in evidence; this represents a variable amount of residual unadsorbed phage, which conveniently serves as a reference for parental density.

FIG. 1. CsCl analysis of progeny phage. Progeny phage were added to 10/9 CsCl (A. W. Kozinski, *Virology* **13**:124, 1961) and centrifuged for 20 hr at 27,000 rev/min at 10°C in an SW39 head. Approximately 25 fractions were collected in shell vials; 10 μ liters was transferred from each fraction to fiber-glass filters for estimation of radioactivity. Another sample was diluted stepwise in 3 M KCl-nutrient broth, to avoid possible osmotic shock, and was plated on B23. The area between the lower arrows was isolated, dialyzed against saline-citrate, and used for extraction of DNA. Note the close proximity of P^{32} to the plating peak.

The distance separating heavy and light references in a CsCl density gradient is shorter for T7 than for T4. This is due to the (A + T):(G + C) ratio of the phage DNA; T7 has a lower thymine content than does T4 (E. Volkin et al., *Virology* **6**:545, 1958). The percentage displacement of the parental ^{32}P from the plating peak of

FIG. 2. CsCl analysis of DNA extracted from progeny phage. (A) Progeny phage were isolated as described in Fig. 1, and native DNA was extracted with phenol; it was supplemented with H^3 reference DNA. The mixture was added in 0.3 ml to 3.0 ml of 9.3 molar CsCl and centrifuged at 30,000 rev/min for 72 hr at 10°C in an SW39 head. The gradients were collected in shell vials. The optical density of each fraction was measured at 260 $m\mu$; the fraction was precipitated with 0.3 M trichloroacetic acid in the presence of carrier albumin. The precipitate was dissolved in 1 M NH_4OH , transferred to a glass-fiber filter, dried, and counted in a scintillation spectrometer. (B) Denatured progeny DNA was obtained by incubating extracted DNA with alkali. It was dialyzed against CSF, centrifuged in CSF-CsCl, collected, and analyzed as above. (○) Summed percentages of recovered activity of P^{32} . (□) Summed percentages of recovered activity of H^3 .

the majority of the heavy progeny phage leads to the estimate that, for T7, the parental contribution to progeny phage is about 17% of the phage molecule. This is approximately three times the percentage characteristic of T4. However, since the T7 DNA is only one-fifth as large as T4 DNA (25×10^6 versus 130×10^6 ; Ritchie et al., *J. Mol. Biol.* 23:365, 1967), the actual sizes of the parental pieces are not strikingly different.

To examine the DNA of the recombinant phage, progeny phage were isolated from CsCl density gradients, dialyzed against saline-citrate buffer, and extracted with phenol; the DNA was banded in a CsCl density gradient in native or denatured form. (DNA was denatured by incubation in saline-citrate containing 0.2 M NaOH for 5 min at 37 C.) The mixture was then dialyzed against saline-citrate containing 1% formaldehyde and banded in CsCl containing 1% formaldehyde (CSF-CsCl). Light, ^3H -labeled T7 DNA, native or denatured, was added to the gradients as a reference. The gradient fractions were collected in vials, and the optical density of the samples was measured at 260 $m\mu$. The samples then were precipitated with trichloroacetic acid, transferred to glass-fiber filter papers, dried, and counted in a scintillation spectrometer. For native DNA (Fig. 2 A), the percentage displacement of the parental ^{32}P from the optical density of the bulk of heavy material was very similar to the percentage displacement of the recombinant progeny from the plating peak of the majority of progeny phage. This eliminates the possibility that two, or more, separate DNA molecules of different densities had been incorporated into the phage head. When the

progeny DNA was melted by alkali (Fig. 2 B, before analysis in CSF-CsCl density gradients) the parental ^{32}P was shifted further away from the heavy optical density reference, but it did not return completely to the light, denatured, ^3H location. This proves that most of the parental fragments are covalently bonded to the heavy progeny molecule; i.e., the progeny, heavy polynucleotide chain is "repaired" at the junction with the parental, light radioactive piece. Because of the proximity of the light and heavy peaks, it is difficult to exclude a certain minority of unrepaired fragments. When progeny DNA in the native form is sonically treated prior to CsCl density gradient analysis, the parental ^{32}P is found, after banding, in a hybrid position, i.e., half way between the heavy reference of optical density and the light reference of ^3H .

In summary, like T4, parental T7 phage DNA recombines with progeny DNA, yielding a population of progeny DNA molecules containing parental fragments; the parental contribution per progeny molecule which receives a fragment is, on the average, 17% of the T7 phage DNA molecule. Since, in contrast to T4, T7 is not circularly permuted (though, like T4, it is terminally redundant; Ritchie et al., *J. Mol. Biol.* 23:365, 1967), the present results leave room for speculation concerning the possible role of recombination in generating circularly permuted molecules.

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