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Intracellular Fate of Deoxyribonucleic Acid from T7 Bacteriophages

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Bacteriophage deoxyribonucleic acid (DNA) molecules may be circularly permuted, as in the T-even phages, or they may have unique nucleotide sequences, as in T7. Both types are terminally redundant (14). In T4 phages, an intermediate replicative form is found, at late times after infection, the strands of which are longer than those of the mature phage (3). Since the appearance of these longer strands coincides with parent to progeny recombination, it may be reasonably assumed that they arise from recombination followed by covalent repair of the gaps in the polynucleotide chains. Recombination as a means of generating long molecules has been postulated by Streisinger et al. (13) and Kozinski et al. (8). A population of circularly permuted and terminally redundant molecules may result if the long intermediate form is cut at equally spaced intervals, somewhat longer than one genome equivalent in length.

Parent to progeny recombination similar to that described for T4 (4, 6) is found in T7 (10). However, the molecular structure of the replicative and recombinant form of parental T7 DNA has not been analyzed. The results of analyses of replicative and recombinant T7 DNA in both native and denatured form, as well as evidence for the participation of one or both strands in transfer to the progeny, are necessary to discriminate between the several possible ways in which a population of unique molecules may be generated.

If both parents and progeny are fragmented prior to recombination, there are two obvious modes of reassembly: the recombinant molecule may be of the same size as the mature molecule or larger. A larger structure may then either undergo repair after recombination or retain single-stranded unrepaired gaps. Gaps located one genome apart could serve as position markers for a diagonal cutting mechanism which, after synthetic repair at the diagonally cut ends, would produce a population of terminally redundant, though not circularly permuted, molecules. It is quite likely that there is a genetic mechanism determining the existence of unrepaired breaks at unique places in the DNA molecule in view of the fact that single-stranded nicks are located at unique positions in the mature T5 DNA molecule (1).

On the other hand, fragmentation may be restricted specifically to parental DNA. In this case, progeny strands would not participate in exchanges but could serve as molds on which the parental fragments are assembled, yielding unique molecules upon repair. This could be achieved if only one parental strand is fragmented, while the other remains integral and replicates semiconservatively. Since no intact parental strands are found in the progeny (10), the replicating strand would not be transferred. A similar mechanism has been observed in phage ϕX 174; in this phage, the parental strand is not transferred to the progeny (5, 12). Transfer of only one parental strand has also been suggested for T4 on the basis of genetic evidence (11, 15). However, this hypothesis is not sustained by data presented here.

The purpose of this paper is to analyze the integrity of replicating and recombining T7 DNA in native as well as in denatured form, and to provide evidence for the transfer of one or both parental strands to the progeny.

The media and analytical procedures were similar to those described by Kozinski et al. (8). Isotope-labeled phages were prepared in *Escherichia coli* B96, and *E. coli* B23 was used for experiments. Phages were purified in the following way. Bacterial debris was removed by centrifuging the lysate at 5,000 $\times$ *g* for 10 min in a Sorvall SPX rotor. Phages were then sedimented at 60,000 $\times$ *g* for 30 min in a Spinco 40 rotor and were washed once; the final suspension was spun once more at 5,000 $\times$ *g* for 10 min. Recovery of viable phages was 60 to 70%.

Bacteria in heavy (5-bromodeoxyuridine-substituted) medium were infected with light (not substituted) ^{32}P-labeled phages, and DNA was extracted from the cells after infection. This DNA was supplemented with light, ^{3}H-labeled DNA and was analyzed in a cesium chloride gradient. It was found that only part of the adsorbed labeled DNA banded at densities higher than the reference. A large fraction of the label, which will be

referred to as conservative, was invariably found at the parental density, decreasing from 90% of the total amount at 4 to 5 min after infection to 60 to 70% at the time of the first burst. In the purified progeny DNA, 30 to 50% conservative material was observed, compared to the 10% reported by Miller (10). Repeated washing of the infected cells did not reduce the conservative moiety in the progeny.

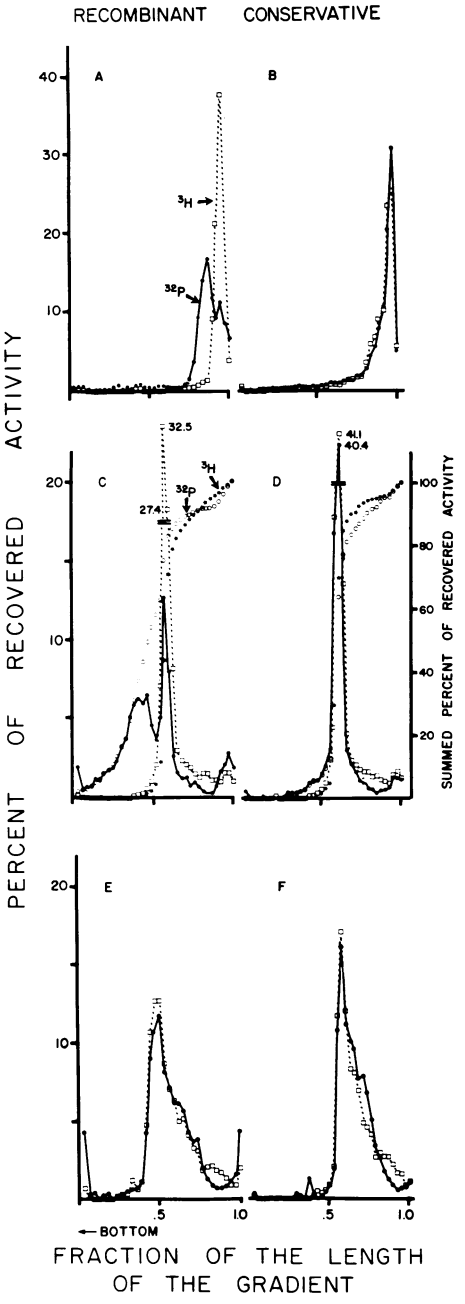

FIG. 1. *Cesium chloride and sucrose gradient analysis of isolated recombinant and conservative moieties*

Conservative and recombinant fractions of intracellular DNA were then isolated and, after dialysis, reanalyzed in cesium chloride and in neutral and alkaline sucrose gradients (Fig. 1). ^{3}H-labeled DNA from light phages was added as a reference. The zone sedimentation profiles of conservative native (Fig. 1D) and denatured (Fig. 1F) DNA largely coincided with those of the mature DNA. In contrast, a large proportion of the recombinant moiety, when analyzed in a neutral sucrose gradient, sedimented faster than the reference integral molecules (Fig. 1C). The rate corresponded to a molecule 2 to 3 phage equivalent units long [calculated according to E. Burgi and A. D. Hershey (2)]. Significantly, the sucrose sedimentation pattern of this moiety after denaturation was identical to that of the reference DNA (Fig. 1E). These results are in agreement with those of Kelly (T. J. Kelly, Federation Proc., p. 591, 1968), who analyzed newly synthesized ^{3}H-labeled T7 DNA in sucrose gradients.

Evidence for the transfer of one or both parental strands to the progeny in T7 was obtained by use of polyribonucleotide complementation as a tool for the separation of the two DNA strands in a density gradient (9). In addition, T4 progeny were analyzed. In this phage, both strands replicate (7), but there has been no evi-

of T7 DNA. Heavy E. coli was infected with light ^{32}P-labeled (specific activity 20 mc/mg) phage (multiplicity of infection = 10), and a sample was removed after 6 min. Native DNA was extracted and banded in CsC; no reference DNA was added. Fractions were collected in shell vials, and 10 μliters of each was transferred to glass-fiber filter papers for counting. Recombinant and conservative groups of fractions were then pooled and dialyzed against citrate-salt, fresh reference DNA from ^{3}H-labeled phages was added, and the DNA mixtures were used for reanalysis in cesium chloride and sucrose gradients. (A, B) Cesium chloride analysis. A 0.3-ml amount of the DNA mixture was added to 3.0 ml of 9.3 M CsCl in citrate-salt, overlaid with mineral oil, and centrifuged for 3 days at 15 C and 55,000 × g in a Spinco SW 39 rotor. Fractions were collected on glass-fiber filter papers, and isotope distribution was measured in a Packard Tri-Carb liquid scintillation spectrometer. (C-F) Sucrose analysis. A 0.2-ml amount of the DNA mixture was layered on 5 to 20% sucrose prepared in 1 M NaCl (neutral gradients, C and D) or 1 M NaCl-0.2 M NaOH (alkaline gradients, E and F). The gradients were centrifuged for 3 hr at 15 C and 100,000 × g in a Spinco SW rotor. Fractions were collected and counted as described above.

dence that both strands are also transferred to the progeny. This is an important point in discriminating between several possible modes of DNA replication.

Light *E. coli* was infected with ^{32}P-labeled T7 or T4, and the progeny were isolated; DNA was extracted, supplemented with proper ^{3}H-labeled reference DNA, complemented with polyuridine and polyguanidine (Miles Laboratories, Inc.; poly-UG, 1:1), and banded in cesium chloride according to Kubinski et al. (9) (*see* legend, Fig. 2).

If both parental strands were transferred equally to the progeny, ^{32}P would produce two peaks overlapping those of the reference ^{3}H-DNA. Transfer of one strand would result in only a single ^{32}P-peak, coinciding with one of the two reference peaks. Unreplicated parental phage contribute negligible amounts to the total transferred activity in T4 (6). In T7, on the other hand, up to one half of the ^{32}P transferred to the progeny may be accounted for by unreplicated parental phage. This fraction is equally distributed between two peaks. Thus, transfer of a single strand in the poly-UG test in the case of T7 would lead to a density profile with at least 75% of the ^{32}P in one peak and at most 25% in the other, compared to the equal distribution of reference-^{3}H between the peaks. However, the observed distribution of both isotopes in the gradient is identical (Fig. 2). This is true for both T7 and T4 (note the overlapping integral curves), indicating that both parental ^{32}P-labeled strands are transferred to the progeny to the same extent in these phages. In either case (T7 or T4), transfer of parental label to both progeny strands could be nonspecific; i.e., due to the breakdown of parental DNA to free nucleotides and random reincorporation of these into the progeny molecules. This is contradicted, however, by the fact that the parental contribution to the progeny molecules is confined in a relatively large subunit of continuous parental origin (4, 10).

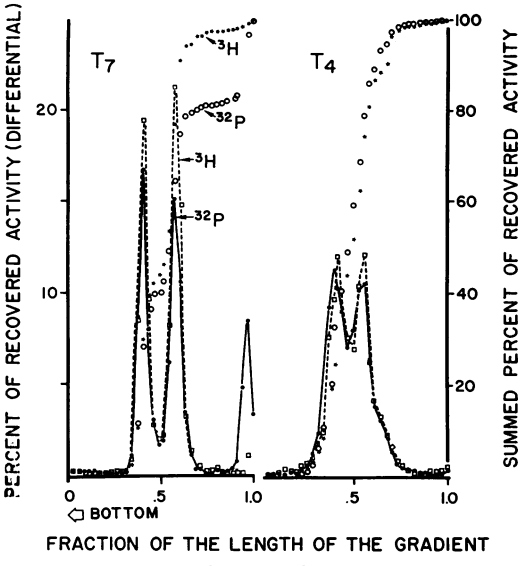

FIG. 2. *Cesium chloride analysis of denatured DNA from the progeny of ^{32}P-labeled T7 and T4 phages after complementation to poly-UG, as compared to reference ^{3}H-DNA. Bacteria were grown to 3 × 10^{8} cells/ml and were infected with T7 (specific activity, 5 mc/mg; multiplicity of infection = 10) or T4 (specific activity, 5 mc/mg; multiplicity of infection = 3) phages. After 40 and 60 min, respectively, the progeny phages were purified; DNA was extracted with phenol, supplemented with light, ^{3}H-labeled DNA and with DNA from cold phage, denatured with alkali, and dialyzed against 1 mM ethylenediaminetetraacetic acid (EDTA). To 0.35 ml of this mixture, containing 10^{10} to 10^{11} phage equivalent units of DNA, 150 μg of poly-UG in 0.04 ml was added; the samples were heated to 95 C for 2 min and rapidly chilled. A 0.35 ml amount of 1 mM EDTA and 2.6 ml of saturated CsCl were added, and the solution was centrifuged for 3 days at 15 C and 70,000 × g in a Spinco SW 39 rotor. Fractions were collected and counted as described in legend to Fig. 1.*

In summary, native replicating and recombining T7 DNA from infected cells sediments through sucrose gradients at a rate corresponding to a molecule 2 to 3 times longer than one phage equivalent unit. The sedimentation rate after denaturation, however, resembles that of mature DNA, indicating the presence of single-stranded nicks about one phage equivalent unit apart.

Both strands in T7 and T4 are transferred to the progeny with equal probability. The occurrence of single-stranded transfer in T4 phages, as suggested by Barricelli and co-workers (11, 15), is therefore not sustained by the present findings. Transfer of both strands to the progeny, together with the possible formation of a long intermediate form containing single-stranded nicks one phage equivalent unit apart, rules out the hypothesis that intact progeny strands act as receptacles for fragments of parental strands. It raises the possibility that the unique T7 DNA molecules result from uniquely located nicks, determining a unique mode of cutting the molecule. Further elaboration of this mechanism demands more extensive discussion elsewhere.

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