NOTES

Equal Incorporation of Both Parental Bacteriophage T7 Deoxyribonucleic Acid Strands into Intracellular Concatemeric Deoxyribonucleic Acid

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After infection of *Escherichia coli* B with radiolabeled T7 bacteriophage, the parental deoxyribonucleic acid label was found in both polynucleotide chains of the intracellular T7 concatemer.

After infection of *Escherichia coli* B by bacteriophage T7, newly synthesized phage deoxyribonucleic acid (DNA) is found in a long linear structure called a concatemer. The concatemer contains several T7 phage genomes presumably united head to tail. Later in the infectious cycle, this concatemer is specifically cleaved to produce nonpermuted, mature phage DNA molecules, each of which is of unit length bearing terminal repetitions of 200 to 300 nucleotide pairs (1, 3, 10).

Kelly and Thomas (5) suggested that this concatemeric intermediate could be produced either by the joining of newly made molecules by genetic recombination between the repetitious ends or directly by DNA replication from a circular structure. Gilbert and Dressler (2) proposed a model, termed the rolling circle model, in which one of the parental strands is displaced from the circle by newly replicated DNA at the replication fork, although the other parental strand remains circular. Replication then proceeds around the circle many times, displacing a linear DNA molecule whose length could be much longer than that of the original mature-sized DNA molecule.

Two examples are known which support this model: the synthesis of single-stranded ϕ X 174 DNA (2, 6) and the transfer of F DNA from donors to recipients during bacterial mating in *E. coli* (4, 8, 9, 11; D. Vapnek and W. D. Rupp, *J. Mol. Biol.*, *in press*).

If DNA replication proceeds in only one direction with regard to the gene order, the fate of the two parental strands in this model is quite different. One parental strand would be expected to remain circular and have a length essentially equal to mature-sized DNA. The other strand would be displaced into the concatemer and hence be part of a DNA molecule having a length greater than that of mature DNA. This leads to the prediction, which we sought to test, that only one of the two parental strands should be recovered in the concatemer. Our results show the contrary. Radioactive label from parental DNA can be recovered in both strands of the concatemer. This result thus extends the earlier reports that radioactive label in parental DNA of either phage T7 or lambda is recovered equally in both DNA strands of the progeny phage particles that are recovered after one cycle of growth (1, 4, 12).

Kelly and Thomas (5) found that pulse label readily entered the rapidly sedimenting fractions that constitute concatemer. In the initial experiments, we found little or no parental label sedimenting more rapidly than mature-sized T7 DNA. However, when chloramphenicol (100 μ g/ml) was added at 7 min after infection (at which time T7 DNA synthesis begins), as much as 30% of the parental label could be recovered in fractions sedimenting faster than mature T7 DNA at the same position as newly replicated DNA (Fig 1). Presumably, the chloramphenicol prevents synthesis of the proteins concerned with phage maturation and breakdown of the concatemer (2).

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FIG. 1. *Escherichia coli* strain B, growing exponentially at 2×10^8 bacteria per ml in TCG, (5) was infected with ^{32}P -labeled T7 phage at a multiplicity of 10 phage per bacterium in a volume of 10 ml. Seven minutes after infection chloramphenicol was added to 100 $\mu\text{g}/\text{ml}$, and ^3H -thymidine (10 μCi) was added. At 9 min after infection, an equal volume of ice-cold solution containing 0.1 M ethylenediaminetetraacetic acid (EDTA; pH 7.0) and 0.005 M KCN was added, and the bacteria were centrifuged. After resuspension in the same solution, the bacteria were centrifuged again and resuspended in 1 ml of solution. The bacteria were then lysed by the method of Kelley and Thomas (5). Sodium lauryl sulfate was added to a concentration of 1%, and, after 10 min at 37 C, Pronase (preincubated 15 min at 37 C) was added to a concentration of 1 mg/ml. The lysate was incubated for 6 hr at 37 C and overnight at room temperature. A sample of the extract was layered onto a 5-ml 30 to 10% glycerol gradient containing 0.01 M tris(hydroxymethyl) aminomethane buffer (pH 7.4), 0.001 M EDTA, and 0.02 M NaCl and centrifuged for 1 hr at 48,000 rev/min in an SW 50 rotor. Symbols: $\bullet$, ^{32}P -parental DNA; $\circ$, ^3H -pulse-labeled DNA.

The concatemer DNA was pooled and resedimented through a second glycerol gradient to establish that rapid sedimentation was not an artifact of the isolation procedure; the profiles were not altered by dilution, by phenol extraction, or by simple aging for 2 weeks at 4 C. Though neutral gradient sedimentation is not always a reliable indication of the molecular length of replicating DNA (7), Kelly and Thomas (5) previously demonstrated a fair agreement between molecular lengths of the concatemers observed in the electron microscope and the distribution of lengths predicted from the sedimentation profiles.

The concatemeric DNA containing both pulse and parental labels was denatured with 0.2 M NaOH, reneutralized, mixed with poly rU,G, and sedimented to equilibrium in a CsCl gradient. This procedure separates the two strands of DNA if they contain unequal numbers of poly rU,G-binding sites (13). Phage T7

has the fortunate property that most or all of the binding sites are located on one strand (13), thus allowing clean separation of the T7 strands even though they may be of variable length. Both parental and pulse label were found to be present equally in the two complementary strands (Fig. 2). A similar result was obtained when mature phage DNA was used as a marker instead of newly replicated DNA. From this experiment, we conclude that parental label from both strands is transferred into concatemers.

To confirm this conclusion, the foregoing experiment was repeated with single-stranded concatemeric DNA rather than double-stranded concatemer. The infected cells were lysed directly in 0.2 M NaOH and the lysate was sedimented through an alkaline gradient. Some of the parental label sedimented more rapidly than mature-sized single strands derived from mature phage particles (Fig. 3). The leading edge of the peak (Fig. 3) was pooled and resedimented through a neutral glycerol gradient containing 1 M NaCl to separate further the longer strands from those of mature size. This DNA was complexed

FIG. 2. Concatemeric DNA, obtained by pooling fractions 15 to 17 (Fig 1), was denatured in 0.2 M NaOH for 5 min at room temperature. The DNA solution was neutralized with 0.05 M tris(hydroxymethyl) aminomethane buffer (pH 7.9) containing enough HCl to neutralize the NaOH and was diluted several-fold. An excess of poly rU,G (Miles Laboratories, Inc., Elkhart, Ind.) was added, followed by solid CsCl to a refractive index of 1.4060. The mixture was centrifuged in a fixed-angle 60 rotor for 60 hr at 35,000 rev/min at 18 C. Symbols: $\bullet$, ^{32}P parental label concatemeric DNA; $\circ$, ^3H pulse-labeled concatemeric DNA.

FIG. 3. Cells infected with ^{32}P -labeled phage were centrifuged in ethylenediaminetetraacetic acid (EDTA)-KCN solution as in Fig. 1. After resuspension, NaOH was added to 0.2 N , and Sarkosyl was added to 0.2%. ^3H -labeled marker DNA was added as phage particles directly to the lysate, which was then sedimented in a 30 to 10% glycerol gradient containing 0.2 N NaOH, 0.8 M NaCl, and 0.001 M EDTA for 75 min at 48,000 rev/min in an SW 50 rotor. Symbols: $\bullet$, ^{32}P -labeled intracellular DNA; $\circ$, ^3H -labeled mature marker DNA.

with poly rU,G and banded in CsCl by using denatured DNA from mature phage particles as a marker. The parental label was found about equally in the two bands (Fig. 4). Other experiments showed that pulse label in DNA isolated in a similar way is also distributed equally in the two peaks.

The results shown here demonstrate that intracellular DNA molecules, isolated either before or after denaturation, which are longer than those obtained from phage particles, contain parental and pulse-labeled DNA equally in both kinds of strands. Had label been incorporated unequally into concatemeric DNA, we would have obtained evidence that some strand-specific event was involved in DNA replication or phage maturation. The observed results are consistent, however, with a number of possibilities. For example, parental label may be incorporated into concatemers by recombination or even by degradation and resynthesis. If replication is directly responsible, one must picture some mechanism that extends both kinds of DNA strands.

FIG. 4. Fractions (6 to 11 inclusive) from the experiment outlined in Fig. 3 were pooled and dialyzed against 0.01 M tris (hydroxymethyl) aminomethane (Tris) buffer (pH 7.4), 0.1 M NaCl, and 0.001 M ethylenediaminetetraacetic acid (EDTA). Long strands in this DNA were further purified from mature-sized DNA by resedimentation in a neutral 30 to 10% glycerol gradient containing 0.02 M Tris buffer (pH 7.4), 1 M NaCl, and 0.001 M EDTA for 75 min in an SW 41 rotor at 40,000 rev/min. Fractions sedimenting ahead of the mature-sized marker DNA were pooled and mixed with denatured ^3H -DNA obtained from mature phage particles. Poly rU, G was added and the solution was centrifuged to equilibrium in CsCl as described in Fig. 2. Symbols: $\bullet$, ^{32}P -parental label in long DNA strands; $\circ$, ^3H -marker DNA from mature phage particles.

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