NOTES

Defective Deoxyribonucleic Acid Replication of T4rII Bacteriophage in Lambda-Lysogenic Host Cells¹

RAYMONDE SZARGEL AND CHANNA SHALITIN

Department of Biology, Technion-Israel Institute of Technology, Haifa, Israel

Received for publication 25 April 1972

Sedimentation of the replicative deoxyribonucleic acid through alkaline sucrose gradients showed that rII single chains reached the half-mature size at a time when wild-type molecules formed long chains (dimers and trimers of genome size). Long rII single chains could be observed on substitution of tris(hydroxymethyl)amino-methane buffer for Na⁺K⁺ phosphate in the growth medium.

When lambda lysogens producing a gene product called rex (8) are infected with T4rII mutants, phage development is blocked 10 min postinfection in the presence of K⁺ and Na⁺ ions (6, 17). It has been suggested that the rII product is indirectly involved in the control of an endonuclease activity (2, 10, 11, 13).

The present study was undertaken with a view to understanding the nature of the block of deoxyribonucleic acid (DNA) replication in rII-infected G(λ) cells (1), and the influence of the growth medium on replication intermediates was investigated accordingly.

Since tris(hydroxymethyl)aminomethane (Tris) cations were found to support normal growth of rII mutants in lambda lysogens (17), Tris-glucose medium with a low Na⁺ concentration (8.6 mM) and a relatively high concentration of Mg²⁺ ions (1.0 mM) will hereinafter be used as permissive medium (A), whereas M9 medium containing 177 mM Na⁺, 32 mM K⁺, and 0.1 mM Mg²⁺ ions will be used as restrictive medium (B).

The wild-type T4B and the rII mutant of phage T4B, r196 (a small deletion in the B cistron), were obtained from S. Benzer through F. W. Stahl.

Our results show the production of 0.18 plaque-forming units (PFU) of r196 per *Escherichia coli* G(λ) cell (at 50 min and 37°C) under restrictive growth conditions as against 21 PFU/

cell under permissive conditions. Wild-type phage produced about 100 PFU/cell under the same experimental conditions. Qualitatively similar results have been previously reported (6).

Previous experiments (6, 16, 17) have shown that lambda lysogens infected by rII mutants exhibit arrested DNA synthesis 10 to 12 min postinfection. The following experiments were undertaken to determine the rate of synthesis under restrictive conditions. At different times after infection, 1-ml samples of the infected thymidine culture in 250 μg of deoxyadenosine per ml were added to 0.2 ml of thymidine-³H (5 μCi/ml). After 2 min, the reaction was arrested with 2 ml of 10% cold trichloroacetic acid containing 1.8 mg of cold thymidine per ml. The precipitates were collected on membrane filters (Sartorius; 25 mm diameter, 0.45 μm pore size) and were counted as described previously (18). Results (Fig. 1) show that the r196-infected G(λ) cells already synthesize DNA at a reduced rate (by a factor of 2) at 6 min postinfection. Later from 10 min postinfection, rII-infected cells produced DNA at a fairly constant rate, whereas in wild-type infected cells the rate of synthesis is increased up to 20 min postinfection. It is probable that the decreased rate of thymidine labeling does not reflect expanded thymine nucleotide pools since synthesis of DNA by rII-infected cells shows abnormality also as measured by incorporation of ³²P into the DNA (6, 17).

The reduced rate of rII-DNA synthesis as demonstrated in Fig. 1 could be the result of

¹ These results are part of a thesis submitted by R. Szargel in partial fulfillment of the requirements of the M.Sc. degree at the Department of Chemistry, Technion-Israel Institute of Technology, Haifa, Israel.

FIG. 1. Incorporation of thymidine-methyl- ^3H into DNA in medium A versus B (permissive versus restrictive, respectively). *E. coli* G(λ) cells were grown to a density of 2×10^8 cells per ml in medium A. Cells were washed by centrifugation, resuspended at a concentration of 2×10^8 , and infected (multiplicity of infection = 5) with wild-type phage ($\blacktriangle$) or with mutant r196 ($\triangle$) for 2 min at 26°C. In parallel, cells were grown in medium B and similarly infected with wild-type phage ($\bullet$) or with mutant r196 ($\circ$). At zero time, infected bacteria were diluted 10-fold into appropriate growth tubes supplemented with 250 μg of deoxyadenosine per ml. Pulse labeling with thymidine-methyl- ^3H and extent of incorporation is described in text. The time of each point is the middle of the 2-min pulse period.

viral DNA breakdown following rII infection of G(λ) cells. Therefore, the stability of the newly synthesized DNA was determined. A pulse of ^3H -thymidine (1 $\mu\text{Ci}/\text{ml}$) was given at 6 min postinfection and terminated at 10 min with a large excess (10,000-fold) of cold thymidine. Samples (0.5 ml) were withdrawn at different times after infection into 2.5 ml of 6% cold trichloroacetic acid containing 1 mg of cold thymidine per ml, and the radioactivity retained in the acid-insoluble fraction was determined. Our results clearly show no acid solubilization of the DNA synthesized 6 to 10 min postinfection in rII as in wild-type infected cells (*data not shown*).

It was plausible to assume that the abnormal synthesis of DNA demonstrated in Fig. 1 might be related to the intrinsic structure of the DNA. Therefore, zone sedimentation of parental and newly replicated DNA through sucrose gradients was done as described previously (18). Molecular weights for denatured DNA in alkaline gradients were also calculated as described previously (18). Results (Fig. 2A) clearly show that at an early stage postinfection (8 min), both parental rII and r^+ single strands cosediment with whole reference molecules. In contrast

to parental DNA, progeny single-stranded DNA, synthesized at the same time postinfection (Fig. 2C), sediments at a rate corresponding to shorter than mature reference molecules. rII molecules have, on the average, 2.3 to 7 nicks per single strand, whereas r^+ molecules are relatively longer but also contain 1.3 nicks per single strand on the average. (The latter value is in accord with previous observations [5].) Parental and progeny DNA show similar sedimentation profiles at a late stage postinfection (14 min). As seen in Fig. 2B and D, 62% of the r^+ DNA sediments at a rate corresponding to long single strands (exceeding one genome length), as compared to only 26% of the rII DNA. For most of the latter, the sedimentation rate corresponds to half-molecules on the average, whereas for the former it corresponds to linear monomers, dimers, and trimers of genome size. The conclusion is that, in contrast to denatured parental DNA, newly synthesized denatured rII DNA is shorter than its wild-type counterpart.

In view of the interruptions observed in single-stranded progeny rII DNA (Fig. 2D), it was of interest to determine whether two-strand cuts could be detected in duplex DNA. Comparative sedimentation analysis of r^+ and rII progeny DNA (labeled 12 to 16 min postinfection) through neutral sucrose gradients, indicated (*data not shown*) that both types sedimented as $\sim 200\text{S}$ replicating DNA, as observed previously by Frankel in standard infection (4). Thus, it seems that native rII DNA has no double-stranded interruptions, whereas denatured rII DNA has one nick per single strand (Fig. 2D). Permissive conditions found to reverse the rII phenotype (Fig. 1) are thus expected to suppress the interruptions in denatured rII DNA molecules. In fact, results shown in Fig. 3 demonstrate that progeny rII DNA labeled 12 to 16 min postinfection in a permissive medium sediments through alkaline sucrose gradients at a rate corresponding to monomers, dimers, and trimers of genome size, similar to wild-type DNA (see Fig. 2D). We conclude that in the presence of low Na^+ and K^+ concentrations, rII DNA forms normal long single strands.

The next objective was to determine whether the interruptions observed mainly in newly synthesized rII DNA (see Fig. 2D) are introduced as a result of nuclease action or are inherent properties of the newly replicated DNA (15, 19). Bearing in mind that admixture of chloramphenicol at 5 min postinfection inhibits endonucleolytic breakage of DNA (12, 18) but not formation of short DNA chains (7, 9), the effect of chloramphenicol (30 $\mu\text{g}/\text{ml}$), added at 5, 7, or 9 min postinfection, on the rate of rII DNA synthesis was studied. Experiments similar to

FIG. 2. Sedimentation of ^{14}C -labeled intracellular T4 DNA in alkaline sucrose gradients. *E. coli* G(λ) cells were infected with wild-type phage (●), or with mutant r196 (○) in medium B. For pulse labeling newly replicated DNA, thymidine-2- ^{14}C (1 $\mu\text{Ci}/\text{ml}$) was added at different times postinfection in medium B, supplemented with 250 μg of deoxyadenosine per ml. Label incorporation was arrested by placing a sample in an equal volume of ice-cold 0.1 M KCN. The infected culture was centrifuged and resuspended at a final concentration of 10^9 cells/ml. For lysis, the medium specified previously (18) was added, except that lysozyme was omitted. Sodium dodecyl sulfate (known to inhibit nuclease activity) was added at a final concentration of 0.2%. Sedimentation at 4 C was for 90 min at 35,000 rev/min (SW39 rotor). The position obtained for reference ^{14}C -T4 DNA released from whole phage on top of the gradient was used to calculate the scale of approximate molecular length (in mature phage equivalents) at the top of the graph. A, Parental DNA at 8 min postinfection; ^{14}C input: r^+ = 860 counts/min; r196 = 1,240 counts/min. B, Parental DNA at 14 min postinfection; ^{14}C input: r^+ = 735 counts/min; r196 = 530 counts/min. C, ^{14}C -pulse-labeled DNA synthesized 6 to 10 min postinfection; ^{14}C input: r^+ = 2,300 counts/min; r196 = 1,320 counts/min. D, ^{14}C pulse-labeled DNA synthesized from 12 to 16 min postinfection; ^{14}C input: r^+ = 7,400 counts/min; r196 = 1,020 counts/min. Recoveries of the order of 80 to 100%.

FIG. 3. Analysis of ^{14}C -labeled intracellular rII DNA formed in medium A versus B (permissive versus restrictive, respectively). *E. coli* G(λ) cells were infected with mutant r196. Thymidine-2- ^{14}C (1 $\mu\text{Ci}/\text{ml}$) was added 12 to 16 min postinfection. Preparation of lysates and sedimentation in alkaline sucrose gradients as in the legend to Fig. 2. ^{14}C input: 8,040 counts/min (●); 1,585 counts/min (○). Recoveries, 95 and 81%, respectively.

those described in Fig. 1 showed no difference in the amount of radioactivity incorporated into acid-insoluble material during 2-min pulses in the presence of chloramphenicol and without it (data not shown). This finding is in accord with previous observations (16). Furthermore, sedimentation of newly synthesized rII DNA (labeled 12 to 16 min postinfection, in the presence of chloramphenicol admixed at 7 min postinfection) through alkaline sucrose gradients showed that the resulting rII DNA contained 2.3 nicks per single strand as against 1 nick per single chain on the average without chloramphenicol (data not shown). In other words, the latter does not prevent the occurrence of DNA interruptions.

Based on the sedimentation analysis of DNA formed after infection with rII mutants, we can construct the following sequence of events in the replication of rII DNA under restrictive growth conditions. At an early stage (8 min, Fig. 2C), the DNA is found in strands shorter than mature DNA, and there is no conversion into rapidly sedimenting long single chains (Fig. 2D), as observed for wild-type infected cells. These results may be interpreted as lack

of endonuclease activity (2, 10, 11, 13), which might be required for molecular recombination (3) since long single strands appearing at a late stage (15 min postinfection) are considered by some to be the product of molecular recombination (14). Alternatively, reduced ligase activity due to an extensive leakage of NAD from rII-infected cells (17) might interfere with the formation of long single strands (7, 12).

LITERATURE CITED

- Arber, W., and C. Lataste-Dorolle. 1961. Erweiterung des wirtsbereiches des bakteriophagen lauf *Escherichia coli* B. *Pathol. Microbiol.* 24:1012-1018.
- Berger, H., and A. W. Kozinski. 1969. Suppression of T4D ligase mutations by rIIA and rIIB mutations. *Proc. Nat. Acad. Sci. U.S.A.* 64:897-904.
- Carlson, K., and A. W. Kozinski. 1970. Parent-to-progeny transfer and recombination of T4rII bacteriophage. *J. Virol.* 6:344-352.
- Frankel, F. R. 1966. Studies on the nature of the replicating DNA in *Escherichia coli* infected with certain amber mutants of phage T4. *J. Mol. Biol.* 18:127-143.
- Frankel, F. R. 1968. Evidence for long DNA strands in the replicating pool after T4 infection. *Proc. Nat. Acad. Sci. U.S.A.* 59:131-138.
- Garen, A. 1961. Physiological effects of rII mutations in bacteriophage T4. *Virology* 14:151-163.
- Hosoda, J., and E. Mathews. 1971. DNA replication *in vivo* by polynucleotide-ligase defective mutants of T4. II. Effect of chloramphenicol and mutations in other genes. *J. Mol. Biol.* 55:155-179.
- Howard, B. D. 1967. Phage lambda mutants deficient in rII exclusion. *Science* 158:1588-1589.
- Iwatsuki, N., and R. Okazaki. 1970. Mechanism of DNA chain growth. V. Effect of chloramphenicol on the formation of T4 nascent short DNA chains. *J. Mol. Biol.* 52:37-44.
- Karam, J. D. 1969. DNA replication by phage T4 rII mutants without polynucleotide ligase (gene 30). *Biochem. Biophys. Res. Commun.* 37:416-422.
- Karam, J. D., and B. Barker. 1971. Properties of bacteriophage T4 mutants defective in gene 30 (deoxyribonucleic acid ligase) and the rII gene. *J. Virol.* 7:260-266.
- Kozinski, A. W. 1968. Molecular recombination in the ligase negative T4 amber mutant. *Cold Spring Harbor Symp. Quant. Biol.* 33:375-391.
- Krisch, H. M., D. B. Shah, and H. Berger. 1971. Replication and recombination in ligase-deficient rII bacteriophage T4D. *J. Virol.* 7:491-498.
- Miller, R. C., Jr., A. W. Kozinski, and S. Litwin. 1970. Molecular recombination in T4 bacteriophage deoxyribonucleic acid. III. Formation of long single strands during recombination. *J. Virol.* 5:368-380.
- Okazaki, R., T. Okazaki, K. Sakabe, K. Sugimoto, R. Kainuma, A. Sugino, and N. Iwatsuki. 1968. *In vivo* mechanism of DNA chain growth. *Cold Spring Harbor Symp. Quant. Biol.* 33:129-143.
- Rutberg, B., and L. Rutberg. 1966. Bacteriophage-induced functions in *Escherichia coli* K(λ) infected with rII mutants of bacteriophage T4. *J. Bacteriol.* 91:76-80.
- Segiuchi, M. 1966. Studies on the physiological defect in rII mutants of bacteriophage T4. *J. Mol. Biol.* 16:503-522.
- Shalitin, Ch., and Y. Naot. 1971. Role of gene 46 in bacteriophage T4 deoxyribonucleic acid synthesis. *J. Virol.* 8:142-153.
- Werner, R. 1971. Mechanism of DNA replication. *Nature (London)* 230:570-572.