Characterization of the Defects in Bacteriophage T7 DNA Synthesis During Growth in the *Escherichia coli* Mutant *tsnB*

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The *Escherichia coli* mutant *tsnB* (M. Chamberlin, J. Virol. 14:509-516, 1974) is unable to support the growth of bacteriophage T7, although all classes of phage proteins are produced and the host is killed by the infection. During growth in this mutant host, the rate of phage DNA synthesis is reduced and the DNA is not packaged into stable, phagelike particles. The replicating DNA forms concatemers but the very large replicative intermediates (~440S) identified by Paetkau et al. (J. Virol. 22:130-141, 1977) are not detected in T7⁺-infected *tsnB* cells. These large structures are formed in *tsnB* cells infected with a T7 gene 3 (endonuclease) mutant, where normal processing of the large intermediates into shorter concatemers is blocked. At later times during infection of *tsnB* cells, the replicating DNA accumulates in molecules about 30% shorter than unit length. Analysis of this DNA with a restriction endonuclease indicates that it is missing sequences from the ends (particularly the left end) of the genome. The loss of these specific sequences does not occur during infections with T7 gene 10 (head protein) or gene 19 (maturation protein) mutants. This suggests that the processing of concatemers into unit-length DNA molecules may occur normally in T7-infected *tsnB* cells and that the shortened DNA arises from exonucleolytic degradation of the mature DNA molecules. These results are discussed in relation to our recent observation (M. A. DeWynngaert and D. C. Hinkle, J. Biol. Chem. 254:11247-11253, 1979) that *E. coli tsnB* produces an altered RNA polymerase which is resistance to inhibition by the T7 gene 2 protein.

The isolation and characterization of bacterial mutants which are defective in their ability to propagate bacteriophage have often been fruitful in elucidating the role of bacterial proteins in phage development. Several bacterial mutants have been isolated which grow normally but which are unable to support the intracellular growth of T7 phage (6, 29, 31). Studies with one of these mutants, *Escherichia coli tsnC*, led to the discovery that *E. coli* thioredoxin is an essential component of the T7 DNA polymerase (15, 19, 20).

We have been studying a second mutant, *tsnB*, with the hope of identifying other host proteins which participate in T7 DNA replication. Chamberlin (6) has shown that *tsnB* adsorbs T7 phage normally and is killed by the infection. The synthesis of T7 RNA and of both early and late classes of T7 proteins and the breakdown of bacterial DNA also occur normally during infection. However, in T7-infected *tsnB* cells, T7 DNA synthesis stops prematurely, shortly after its onset, suggesting that the *tsnB* function affects a step in the late phase of T7 DNA replication.

In this paper, we describe the analysis of T7 DNA synthesized during infection of *E. coli*

tsnB. We show that, although DNA concatemers are formed, the very large replicative intermediates described by Paetkau et al. (21) are not observed. The concatemers formed during this mutant infection are processed abnormally into DNA molecules somewhat shorter than unit length.

These defects closely resemble those which occur during infection of a wild-type cell with a T7 gene 2 mutant. Since the gene 2 protein has been implicated in the "shut off" of early transcription by the bacterial RNA polymerase (8), the results presented in this paper led us to speculate that *E. coli tsnB* might produce an altered RNA polymerase which is resistance to inhibition by the T7 gene 2 protein. We have recently purified RNA polymerase from *E. coli tsnB* and shown that this enzyme is completely resistant to inhibition by purified T7 gene 2 protein (6a). It is not yet clear whether all of the defects in T7 DNA synthesis observed during growth in *E. coli tsnB* can be traced to this defect in the bacterial RNA polymerase. However, the inability to grow T7 phage is greater than 90% cotransducible with *rifA* (*rpoB*) (S. Buchstein and D. Hinkle, unpublished data), suggesting that *tsnB* maps within either *rpoB* or

rpoC, the structural genes for the β and β' subunits of RNA polymerase.

MATERIALS AND METHODS

Bacterial strains and phages. *E. coli* B/1 and the derivative *tsnB* mutant 7009 (6) were provided by M. Chamberlin. T7 phage were from F. W. Studier and were grown as described by Studier (27). T7 mutants are designated by subscript notation indicating gene only. The mutations used are gene 0.7, JS62a (29); gene 2, *am64*; gene 3, *am29*; gene 10, *am13*; gene 19, *am10* (27).

Other materials. Sucrose (ultrapure grade), agarose (electrophoresis grade) and pronase were from Sigma Chemical Co. [^3H]thymidine (20 Ci/mmol) was from New England Nuclear Corp., and carrier-free $^{32}\text{PO}_4$ was from Amersham Corp. Lysozyme was from Worthington, and *HpaI* endonuclease was from Bethesda Research Labs, Inc.

Sedimentation analysis. Neutral sucrose gradients (5 to 20%) were made in 1 M NaCl, 50 mM Tris-hydrochloride, pH 7.5, 1 mM EDTA, and 0.015% Sarkosyl, and were overlaid with a 0.3-ml shelf containing 1.25 g of CsCl per 1 ml of 20% sucrose solution. Centrifugation was at 4°C in an SW 50.1 or an SW 60 rotor as described in the figure legends. Constant-volume (usually 0.2-ml) fractions were collected from a hole in the bottom of the tube by injecting portions of mineral oil through a rubber stopper at the top of the tube with a Cornwall syringe. Acid-insoluble radioactivity in the fractions was determined as described previously (11).

RESULTS

Mutations in T7 gene 0.7 affect the kinetics of T7 DNA synthesis during infection of *tsnB*. Chamberlin has shown that during T7⁺ infection of *tsnB* the kinetics of T7 DNA synthesis is abnormal (6). The rate of DNA synthesis decreases prematurely between 10 and 15 min after infection and then gradually increases again, reaching a maximum between 20 and 25 min. In our initial experiments we were unable to reproduce the Chamberlin results. We found that in T7-infected *tsnB* cells T7 DNA synthesis stopped prematurely between 10 and 15 min and did not begin again. Subsequently, during analysis of our "wild-type" T7 DNA with the restriction endonuclease *HpaI*, we discovered that this phage contained a deletion of about 900 base pairs, apparently encompassing the *HpaI* site at 6.25%, which lies within gene 0.7. This gene codes for a protein kinase which is not essential for growth on normal hosts but which is apparently involved in shutting off host and T7 early RNA synthesis (9, 17). Further experiments, using a true wild-type phage and a gene 0.7 point mutant, confirmed that mutations in T7 gene 0.7, while having no apparent effect on the kinetics of DNA synthesis in a wild-type host

(data not shown), cause a more rapid and dramatic decrease in T7 DNA synthesis in T7-infected *E. coli tsnB* (Fig. 1).

Noninfectious phage particles are not formed in T7-infected *tsnB* cells. Since a substantial amount of T7 DNA is synthesized in T7⁺-infected *tsnB* cells, and since phage RNAs and proteins are synthesized in normal amounts (6), we have tested for the formation of noninfectious phage particles in T7-infected *tsnB* cells. Phage DNA was labeled with [^3H]thymidine at 8 min after infection, and after lysis the [^3H]DNA was analyzed by sedimentation in a sucrose gradient (Fig. 2). In the wild-type control, a substantial amount of the acid-insoluble ^3H sediments with the ^{32}P -labeled T7 phage added as a marker. In contrast, analysis of the lysate from T7-infected *tsnB* cells shows that none of the ^3H -labeled phage DNA is associated with rapidly sedimenting particles. The T7 DNA synthesized during this abortive infection is apparently not packaged into defective phage particles.

Sedimentation analysis of DNA synthesized in pulse-chase experiments. During a normal T7 infection, a significant fraction of pulse-labeled DNA molecules sediment faster than unit-length T7 DNA and have been shown

FIG. 1. Kinetics of DNA synthesis in phage T7-infected cells. Bacteria were grown at 30°C in M9 media (27) to a density of 3×10^8 cells/ml and infected with T7 phage at a multiplicity of 10. At the indicated times after infection, 0.1-ml samples were removed from the culture and incubated for 1 min at 30°C with 0.5 μCi of [^3H]thymidine. Acid-insoluble radioactivity was determined as described previously (12). Symbols: (○) T7⁺-infected *E. coli* B/1; (●) T7⁺-infected *E. coli tsnB*; (■) T7_{0.7}-infected *E. coli tsnB*.

FIG. 2. Assay for noninfectious phage particles after infection of *E. coli tsnB*. Bacteria (5-ml cultures) were grown to a density of 3×10^8 cells/ml at 30°C and infected with T7 at a multiplicity of 10. At 8 min after infection, $5 \mu\text{Ci}$ of [^3H]thymidine was added to each culture, and labeling was continued for 2 min, after which unlabeled thymidine was added to 1 mg/ml. The cells were shaken at 30°C until complete lysis had occurred; the lysate was clarified by centrifugation, and a portion (0.1 ml) was mixed with ^{32}P -labeled T7 phage and analyzed by neutral sucrose gradient sedimentation as described in the text. Centrifugation was for 60 min at 60,000 rpm in an SW 60 rotor. The arrow indicates the position of [^{32}P]T7 phage. Symbols: (●) T7-infected *E. coli* B/1; (○) T7-infected *E. coli tsnB*.

to be linear concatemers, containing several phage equivalents of DNA (7, 12, 24). Pulse-chase experiments have demonstrated that these large replicative intermediates are subsequently processed into unit-length DNA molecules. To determine whether T7 concatemers are formed or processed normally in T7-infected *tsnB* cells, we carried out the experiment shown in Fig. 3. At 9 min after infection, cells were labeled for 1 min with [^3H]thymidine. A sample of the culture was withdrawn for analysis (pulse), and the remaining culture was incubated for 10 min with a large excess of unlabeled thymidine (chase). The DNA was extracted by the procedure of Kelly and Thomas (13) and analyzed by sedimentation on a neutral sucrose gradient. In T7-infected wild-type cells, most of the pulse-labeled DNA sediments more rapidly than the ^{32}P -labeled unit-length T7 DNA, and during the

10-min chase this DNA is converted into unit-length molecules. In T7-infected *tsnB* cells, most of the pulse-labeled DNA is also larger than unit length, although structures sedimenting more than 2.5 times faster than mature DNA (larger than 15 times unit length) are apparently not present. However, during the chase this DNA is converted into DNA molecules which are somewhat shorter than unit length. Thus, although

FIG. 3. Sedimentation analysis of DNA synthesized in pulse-chase experiments. Bacteria were grown and infected with T7 phage as described in the legend to Fig. 1. At 9 min after infection, $25 \mu\text{Ci}$ of [^3H]thymidine was added to a 5-ml culture. At 10 min after infection, a 2.5-ml portion was removed for analysis (pulse), and unlabeled thymidine (final concentration, 2 mg/ml) was added to the remaining 2.5 ml of culture, which was incubated for an additional 10 min (chase). Intracellular DNA was then prepared from these samples by the lysozyme-pronase-sodium lauryl sulfate method of Kelly and Thomas (12), and was mixed with ^{32}P -labeled mature T7 DNA and analyzed by neutral sucrose gradient sedimentation as described in the text. Centrifugation was for 1 h at 60,000 rpm in an SW 60 rotor. The arrow indicates the position of [^{32}P]T7 DNA, and the direction of sedimentation was from right to left. In the sucrose gradients of DNA samples from *E. coli* B/1 (wild type), about 50% more [^3H]DNA was recovered from the chase than from the pulse. This difference was due in part to some continued incorporation of [^3H]thymidine during the chase period, and in part to a lower recovery of pulse-labeled DNA during the extraction procedure.

T7 concatemers are formed during infection of *E. coli tsnB*, the processing of these concatemers is apparently abnormal.

Fast sedimenting complexes are not formed in T7-infected *tsnB* cells. In addition to the concatenated intermediates first identified by Kelly and Thomas (12), which contain up to 10 phage equivalents of DNA, it is now clear that much larger replication complexes are also present in T7-infected cells (1, 3, 10, 21, 25, 26). For example, Paetkau et al. (21) have studied a very fast sedimenting (~440S) complex which is stable to extensive deproteinization and phenol extraction. Visualized with the electron microscope, this complex is a large looped structure which resembles the relaxed *E. coli* nucleoid and apparently contains at least 100 phage equivalents of DNA.

To determine whether these very large replicative intermediates are formed in T7-infected *tsnB* cells, we carried out the experiments shown in Fig. 4. At intervals after infection, cells were pulse-labeled (15 s) with [³H]thymidine, and the intracellular DNA was extracted by the procedure of Paetkau et al. (21) and analyzed by sedimentation in sucrose gradients. In the control experiment (T7-infected wild-type cells), much of the pulse-labeled DNA was in a fast sedimenting form (>200S) which moves to the CsCl shelf at the bottom of the tube. In contrast, during the infection of *tsnB* cells essentially none of this fast sedimenting complex was observed. Although some of this pulse-labeled DNA was in short concatemers, sedimenting up to two times faster than mature T7 DNA molecules, much of it was shorter than unit length.

Similar results were obtained when wild-type and *tsnB* cells were infected with T7 phage bearing a mutation in gene 0.7 (protein kinase) (Fig. 5). The gene 0.7 mutation had no effect on the labeling of fast sedimenting DNA in wild-type cells. In *tsnB* cells, no fast sedimenting DNA could be detected, and most of the pulse-labeled DNA molecules were shorter than unit length.

Effect of mutations in T7 genes 3 and 19 on the sedimentation patterns of pulse-labeled DNA from T7-infected *tsnB* cells. A number of T7 gene products have been implicated in the processing of T7 concatemers into unit-length molecules. Concatemeric DNA (up to 10 phage equivalents long) accumulates during infection with phage mutant in genes 8, 9, 10, 18, or 19 (13, 23, 28). The products of genes 8, 9, and 10 are required for the formation of proheads (23, 28). It seems likely that the products of genes 18 and 19 are involved in some interaction between the prohead and the T7 DNA concatemers, which results in both the

FIG. 4. Sedimentation analysis of pulse-labeled DNA from T7-infected wild-type or *tsnB* cells. *E. coli* B/1 (wild type) or *tsnB* were grown and infected with T7 phage as described in the legend to Fig. 1. At the indicated times after infection, 2.5-ml portions were removed from the culture and incubated for 15 s with 1.25 μ Ci of [³H]thymidine. The DNA was isolated by the procedure of Paetkau et al. (21) and analyzed by neutral sucrose gradient sedimentation as described in the text. Centrifugation was for 2 h at 30,000 rpm in an SW 50.1 rotor. The vertical arrows indicate the position expected for unit-length T7 DNA as determined by sedimentation of [³H]T7 DNA on a parallel gradient. The direction of sedimentation is from right to left.

cutting and packaging of DNA (23, 28). In addition to these proteins the product of T7 gene 3, an endonuclease, is required for DNA processing (8, 21, 26). During infection with gene 3 mutants, the replicating phage DNA remains in the very fast sedimenting (~440S) complex (21).

We have examined the effect of a mutation in gene 3 or gene 19 on the formation of fast sedimenting DNA structures during T7 infection of the mutant host *tsnB*. In the absence of the gene 3 endonuclease, a very fast sedimenting complex is formed during infection of *tsnB* (Fig. 6). In fact, the sedimentation patterns of intracellular DNA pulse-labeled at 9, 15, 21, and 27 min after T7₃ infection of *E. coli tsnB* are essentially identical to the patterns obtained by Paetkau et al. (21) using a wild-type host. At 9 min after infection most of the pulse-labeled DNA is in short concatemers sedimenting slightly faster than unit-length molecules, but by 15 min, pulse-la-

FIG. 5. Sedimentation analysis of pulse-labeled DNA from $T7_{0.7}$ -infected wild-type or *tsnB* cells. The conditions of the experiment, performed with a $T7$ gene 0.7 mutant, are identical to those of Fig. 4.

beled DNA is found in very fast sedimenting structures. Labeling of these structures persists until at least 27 min, and shorter DNA molecules (around unit length) are apparently not produced in significant amounts.

Since the gene 3 endonuclease is involved in the breakdown of host DNA (5), it seemed possible that the very fast sedimenting DNA synthesized during $T7_3$ infection of *E. coli tsnB* represented residual host DNA synthesis. To directly test this possibility, we have determined what fraction of the pulse-labeled DNA is in $T7$ DNA sequence by hybridization to nitrocellulose filters containing $T7$ DNA (Table 1). $T7$ phage [^{32}P]DNA was included in each reaction as an internal control for the efficiency of hybridization. Approximately 90% of the DNA pulse-labeled at either 15 or 27 min after infection was contained in $T7$ sequences and is therefore a product of phage and not host DNA synthesis. Thus, very large $T7$ concatemers are formed during infection of *E. coli tsnB* with a $T7$ gene 3 mutant.

In contrast, very fast sedimenting DNA is not observed during infection of *E. coli tsnB* with a $T7$ gene 19 mutant. The sedimentation patterns of DNA pulse-labeled during this infection (Fig. 7) closely resembles the patterns obtained from $T7^+$ infected *tsnB* cells (Fig. 4) although in $T7_{19}$ -infected wild-type cells, the processing of concatemers to unit-length molecules is apparently defective.

Restriction endonuclease analysis of DNA isolated from $T7$ -infected wild-type

and *tsnB* cells. To further investigate our observation that the processing of concatemers in $T7$ -infected *tsnB* cells leads to the formation of shorter than unit length $T7$ DNA molecules, we have determined the restriction endonuclease *HpaI* digestion pattern of ^{32}P -labeled intracellular DNA. Sedimentation analysis showed that the "short" DNA molecules had a sedimentation coefficient about $90 \pm 5\%$ that of mature $T7$ DNA, suggesting that the DNA was 70 to 80% unit length (2). We wished to determine whether this DNA was missing specific regions of the $T7$ genome or whether the shortening was a random event. The restriction nuclease *HpaI* cleaves mature $T7$ DNA at 18 specific sites, and the map position of each of the digestion fragments has been determined (18). If specific regions of the $T7$ genome were removed from the shortened molecules which accumulate in $T7$ -infected *tsnB* cells, then the *HpaI* digestion pattern of this DNA should be missing specific fragments. To test this possibility, we grew *E. coli* B/1 or *tsnB* in the presence of $^{32}PO_4$ and infected the labeled cells with $T7^+$, $T7_{0.7}$, $T7_{10}$, or $T7_{19}$. At 25 min after infection, the cells were harvested and the intracellular DNA was isolated. After incubation with *HpaI* endonuclease, the digestion frag-

FIG. 6. Sedimentation analysis of pulse-labeled DNA from $T7_3$ -infected *tsnB* cells. The conditions of this experiment, performed with a $T7$ gene 3 mutant, are identical to those of Fig. 4, except that only *E. coli tsnB* was used.

TABLE 1. Analysis of pulse-labeled DNA from T7₃-infected *E. coli tsnB* by DNA-DNA hybridization^a

Input DNA	No. of T7 DNA filters	% Input bound		f _{T7} (³ H/ ³² P)
		³ H	³² P	
³ H T7 + ³² P T7	1	49.7	49.1	1.01
	2	71.7	72.4	0.99
³ H 15 min intracellular + [³² P]T7	1	55.5	62.2	0.89
	2	61.0	72.1	0.85
³ H 27 min intracellular + [³² P]T7	1	56.5	65.1	0.86
	2	74.7	81.4	0.92

^a The fraction of [³H]DNA present in T7 sequences (f_{T7}) was determined by DNA-DNA hybridization (12, 17). Each hybridization reaction contained: either 1 or 2 T7 DNA filters (~30 µg of DNA per filter); a blank filter, 3,500 cpm (0.3 µg) of [³²P]T7 DNA (included as an internal control for efficiency of hybridization); and either 83,400 cpm (1.0 µg) of [³H]T7 DNA, 23,800 cpm of 15 min intracellular [³H]DNA, or 18,300 cpm of 27 min intracellular [³H]DNA. The intracellular DNA was from the samples described in the legend to Fig. 6. Less than 0.2% of the input DNA was bound to blank filters containing no DNA.

FIG. 7. Sedimentation analysis of pulse-labeled DNA from T7₁₉-infected wild-type or *tsnB* cells. The conditions of this experiment, performed with a T7 gene 19 mutant, are identical to those of Fig. 4.

ments obtained from each DNA were separated by electrophoresis and visualized by autoradiography (Fig. 8). The *Hpa*I digestion pattern of DNA purified [³²P]T7⁺ phage was included for comparison.

The *Hpa*I digestion patterns of intracellular T7 DNA isolated from T7⁺- and T7_{0.7}-infected *E. coli tsnB* had reduced amounts of fragments

originating from the left end of the T7 genome. This is particularly clear in the pattern shown for the T7_{0.7}-infected *tsnB* (Fig. 8, fourth from

FIG. 8. *Hpa*I digestion pattern of DNA from T7-infected wild-type and *tsnB* cells. *E. coli* B/1 or *tsnB* were grown at 30°C in TCG media (30) containing 20 µCi of ³²PO₄ per ml to a density of 2.5 × 10⁸ cells per ml. Samples (5 ml each) were infected with T7 phage at a multiplicity of 10. After 24 min, the cells were harvested by centrifugation, and the intracellular DNA was prepared by the lysozyme-sodium lauryl sulfate-pronase method of Kelly and Thomas (12), followed by phenol extraction. Residual phenol was removed by extraction with aqueous ether, and the samples (1 ml) were incubated with 50 µg of RNase A and 25 U of RNase T₁ for 1 h at 37°C. The RNase was removed by phenol extraction, and the samples were dialyzed against two changes of 1 M NaCl-10 mM Tris (pH 7.5)-1 mM EDTA for 12 h and then against 0.1 M NaCl-10 mM Tris (pH 7.5)-1 mM EDTA. A sample (5 to 7 µl, containing 40,000 cpm) of the ³²P-labeled intracellular DNA was incubated with the restriction endonuclease *Hpa*I, and the digestion products were analyzed by electrophoresis on a 1% agarose gel (18). The [³²P]DNA was visualized by autoradiography. The diagram showing the map position of each of the *Hpa*I digestion fragments is from McDonnell et al. (18). The position of each fragment on the gel is indicated at the right of the autoradiogram, and the source of each DNA sample is indicated at the top. T7 wt (wild-type), 0.7, 10, and 19 refer to the genotype of the infecting phage; *tsnB* + and - refer to wild-type (+) or mutant (-) *E. coli*. T7 φ refers to DNA isolated from purified ³²P-labeled T7⁺ phage. The minor bands not specifically identified are partial digestion products due to incomplete cutting of the T7 φ DNA sample.

left), where the fragments F, G, and H are completely absent and the amount of fragment C is greatly reduced. In this pattern, fragment J, which comes from the extreme right end of the T7 genome, may also be missing, although this fragment is difficult to quantitate since it is not well resolved from fragment I. Other fragments from the right end, for example, B, L, M, and N, are clearly present although some appear to be in reduced amounts.

The digestion pattern obtained using DNA from T7 wild-type-infected *E. coli tsnB* (Fig. 8, second from left) also has reduced amounts of fragments F, G, and H, but fragment C does not appear to be affected. This DNA apparently contains some unprocessed concatemers, as indicated by the appearance of a new digestion fragment (labeled F + J) just above fragment B. This fragment, which is difficult to see in this photograph but which can be clearly distinguished in films from shorter exposures, has the mobility expected for the joint fragment formed by recombination at the terminal redundancy (13). If the breakdown into shorter than unit-length DNA molecules is initiated at the ends of mature T7 DNA molecules, then the residual amounts of fragments G and H present in this digestion could arise from concatemers which have not yet been fully processed.

Further evidence that the loss of specific regions of the T7 genome during replication in *tsnB* cells does not occur if concatemers are not first processed into unit-length molecules is shown in the digestion patterns obtained with intracellular DNA from T7₁₀- and T7₁₉-infected cells. As discussed above, the products of the T7 genes 10 and 19 are required for normal DNA maturation. Consistent with this, intracellular DNA isolated from these mutant infections appears to contain no mature T7 DNA molecules, since fragments F and J are completely missing from the digestion pattern and the joint fragment F + J is present in essentially the same intensity as fragment B (Fig. 8, fifth and eighth from left). Most important, with these T7 DNA maturation mutants, the digestion pattern for DNA isolated from infected *tsnB* cells (–) is indistinguishable from that of DNA from infected wild-type cells (+). We do not understand why fragment H appears to be reduced in DNA from T7₁₉-infected wild-type and *tsnB* cells.

Sedimentation patterns of pulse-labeled DNA from T7₂-infected *E. coli* B/1. The sedimentation patterns we have obtained for replicating DNA isolated from T7-infected *tsnB* cells seemed to resemble results obtained with DNA isolated from wild-type cells infected with a T7 gene 2 mutant (4). Using a protocol similar to that described in Fig. 3, Center found that con-

catenated intermediates are not formed in T7₂-infected *E. coli* B and that the replicating T7 DNA accumulates in structures somewhat shorter than unit length (4). To directly compare the effect on T7 DNA synthesis of the phage gene 2 mutation and the host mutation *tsnB*, we have determined the sedimentation patterns of pulse-labeled DNA from T7₂-infected wild-type cells by the procedures (21) used to obtain the data in Fig. 4 through 7. Although some of the DNA pulse-labeled at 9 min after infection sediments faster than unit-length T7 DNA molecules, the very fast sedimenting complex (>200S) is clearly not formed in this mutant infection (Fig. 9).

At later times, most of the pulse-labeled DNA appears slightly shorter than unit length. Thus, the defects in T7 DNA synthesis observed in T7₂-infected wild-type cells closely resemble those in T7⁺-infected *tsnB* cells.

DISCUSSION

The *E. coli* mutant *tsnB* is completely unable to support the growth of phage T7, although all classes of phage proteins are produced and the host is killed by the infection (6). Our studies indicate that, although a large amount of T7 DNA is synthesized during infection of this mutant host, the DNA is not packaged into stable phagelike particles. Some of the replicating DNA is found in concatemers (sedimenting two

FIG. 9. Sedimentation analysis of pulse-labeled DNA from T7₂-infected wild-type cells. The conditions of this experiment, performed with a T7 gene 2 mutant, are identical to those of Fig. 4, except that only *E. coli* B/1 was used.

to three times faster than mature T7 DNA), but the very large replicative intermediates (~440S) identified by Paetkau et al. (21) are not detected in T7-infected *tsnB* cells. However, these large intermediates are observed in T7-infected *tsnB* cells when the normal processing of the large structures into shorter concatemers is blocked by a mutation in the T7 gene 3 endonuclease. Thus, although the *tsnB* mutation appears to affect the stability of large concatemers in the infected cell, it apparently does not block the formation of these intermediates.

At later times during T7 infection of *tsnB* cells, phage DNA accumulates in molecules estimated to be 20 to 30% shorter than unit length. These shortened DNA molecules are missing sequences from the ends (particularly the left end) of the genome, and the loss of these specific sequences does not occur in the absence of the T7 gene 10, or gene 19 proteins, proteins known to be required for normal processing of T7 concatemers (23, 28). This suggests that the processing of concatemers into unit-length DNA molecules may occur normally in the *tsnB* cell and that the shortened DNA arises from exonucleolytic degradation of the mature DNA. For example, since the *tsnB* mutation blocks the formation of stable phage particles, the matured DNA may be left exposed to nucleases. The preferential loss of sequences at the left end of the genome could result because some packaging intermediate protects the right end from degradation.

Our observation that the defects which occur in phage DNA synthesis during infection of *tsnB* cells closely resemble those which occur during infection of a wild-type cell with a T7 gene 2 mutant (4), coupled with the observation that these defects are made more severe by a mutation in the T7 protein kinase (gene 0.7), lead us to speculate that the *tsnB* mutation might produce an altered bacterial RNA polymerase. During a normal T7 infection, transcription by the host RNA polymerase is turned off after about 5 min (8, 16, 22). The products of both T7 gene 0.7 and gene 2 have been implicated in this "host shut off." The protein kinase, which is not required for growth on most hosts, partially inactivates the RNA polymerase (22), presumably through phosphorylation of the enzyme (32). The gene 2 protein binds tightly to the RNA polymerase, resulting in complete inactivation of the enzyme (6a, 9, 14). It seemed possible that the *tsnB* mutant might produce an RNA polymerase which was resistant to inhibition by the T7 gene 2 protein. We have recently purified RNA polymerase from *E. coli tsnB* and confirmed that this enzyme is completely resistant to inhibition by purified T7 gene 2 protein (6a.). We

have also shown that the *tsnB* mutation is greater than 90% cotransducible with *rifA* (*rpoB*), suggesting that the mutation is in either *rpoB* or *rpoC*, the structural genes for the β and β' subunits of RNA polymerase (S. Buchstein and D. Hinkle, unpublished data). Thus, inactivation of the bacterial RNA polymerase is apparently required for late stages of T7 DNA synthesis. Possibly, continued transcription of the T7 early region interferes with the packaging of DNA into mature phage particles.

Yamada et al. (31) have studied a bacterial mutant, Y 49, on which T7 wild-type phage can grow, although poorly, but on which T7 gene 0.7 mutants are restricted. The defects in phage DNA synthesis and packaging during growth on this mutant closely resemble those we have observed during growth on *E. coli tsnB*. We have studied a similar mutant, *E. coli* BR 3 (29), which plates T7⁺ phage but not T7 gene 0.7 mutants. Like *tsnB*, the BR 3 mutation appears to map within *rpoB* or *rpoC* (S. Buchstein and D. Hinkle, unpublished data) and produces an RNA polymerase which is resistant to inhibition by the T7 gene 2 protein (6a). We do not yet understand why T7 wild-type phage can grow, although poorly, on *E. coli* BR 3 but not on *tsnB*. Perhaps, in vivo, the BR 3 RNA polymerase is only partially resistant to the gene 2 protein, so that the combined action of the gene 2 protein and the gene 0.7 protein kinase results in sufficient inactivation of the enzyme to permit phage growth. However, in addition to T7 gene 0.7 mutants, phage-bearing deletions in several other regions of the T7 genome also fail to grow on *E. coli* BR 3 (29), suggesting that several factors may influence the requirement for inactivation of the bacterial RNA polymerase by the gene 2 protein.

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