Deoxyribonucleic Acid Replication in λ Bacteriophage Mutants

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After ultraviolet light induction of *Escherichia coli* K-12 strain W3350(λ), several structural intermediate forms of phage deoxyribonucleic acid (DNA) are synthesized. The early defective lysogens of λ, sus O_8, sus P_3, and T_{11}, were found to synthesize none of the DNA structural intermediates. A lysogen believed to be defective in all known phage activities, λsus N_7, was found to be able to synthesize an early phage DNA intermediate. The lysogen λsus Q_{21}, defective in late phage functions, is able to synthesize the early phage DNA intermediate and a concatenated molecule of greater molecular weight than the mature λ DNA.

The induction of *Escherichia coli*, lysogenic for the phage λ, results in the de novo synthesis of several intermediate forms of phage deoxyribonucleic acid (DNA). The first major intermediate of λ DNA synthesized after lysogenic induction has the sedimentation properties of mature λ DNA (10, 14). However, this early intermediate (Intermediate I) has been shown to lack the normal, single-stranded 5' end found in the mature λ DNA (17). The intermediate serves as a precursor for a large, linear, concatenated molecule of greater molecular weight than the mature λ DNA (14, 17). The DNA concatemers may be similar to the replicative intermediates reported after infection of *E. coli* by the phages T_4, T_5, and λ (7, 15, 16). Before cell lysis and release of mature phage, the large λ concatemers are broken down to the size of mature λ DNA (14, 17).

Another structural form of λ phage DNA, formed in small amount after infection and induction, is the covalently linked circular molecule. The circular phage DNA is apparently not converted to mature phage DNA, and its function is unknown (10, 17).

To learn more about the forms of DNA involved in phage replication, a group of suppressor-sensitive, conditional (sus) mutants, isolated and characterized by Campbell (3), were investigated for their ability to synthesize the DNA intermediates. In a previous report (14), some of the late sus mutants of λ, which synthesize phage DNA but are defective in phage maturation, were studied. All of the late mutants tested synthesize the early intermediate and concatenate forms of phage DNA. Some of the late mutants were found to be defective in the ability to break down the

concatenate to the mature phage DNA (14). In the present study, the early λ mutants sus N_7, sus O_8, sus P_3, and T_{11}, and another mutant which also does not cause cell lysis, λsus Q_{21}, were studied for their ability to synthesize the λ DNA intermediates. Previous work has shown that the sus N, sus O, sus P, and T_{11} mutants are unable to synthesize significant amounts of phage DNA (2, 9). It is thought that the λ sus Q mutants are defective in the control of late phage functions (5).

Several of the intermediates studied, λsus O_8, sus P_3, and T_{11}, were unable to synthesize any of the intermediate structural DNA forms. Other mutants, λsus N_7 and sus Q_{21}, were able to synthesize the early intermediate as well as a concatenated form of DNA which sediments faster in neutral and alkaline sucrose gradients than the mature λ DNA.

MATERIALS AND METHODS

Bacterial strains. E. coli K-12 strain W3350 (S), nonlysogenic; W3350(λ), lysogenic for λ phage; and *E. coli* W3350, lysogenic for the suppressor-sensitive, conditional lethal λ sus mutants, sus N_7, sus O_8, sus P_3, and sus Q_{21}, were the gift of A. Campbell. *E. coli* K-12 strain C600, lysogenic for the absolute defective λ strain T_{11}, was obtained from M. B. Yarmolinsky.

Lysogenic induction. The media used for cell growth and the conditions for lysogenic induction of the bacterial strains by irradiation with ultraviolet light have been previously described (14).

Preparation of pulse-labeled phage DNA. Cultures (50 ml) of an appropriate *E. coli* strain were grown in synthetic medium to a cell density of 2×10^8 cells/ml and then induced with ultraviolet light. At 45, 60, or 70 min after induction, 0.15 mc of ³H-thymidine (New

England Nuclear Corp., 8 c/mmole) was added to a 25-ml sample of induced culture. After 1 min, 1.0 ml of thymidine (50 μmoles/ml) was added. After an additional 5 min of incubation, the culture was rapidly cooled to 0 C and the cells were collected by centrifugation. The cells were lysed by lysozyme and sodium dodecyl sulfate and were then extracted with phenol as previously described (14). Under these experimental conditions, lysis of the wild-type lysogenic cell occurs about 80 min after treatment with ultraviolet light. None of the λ mutants examined here caused host cell lysis after induction by ultraviolet light.

Sucrose gradient centrifugation. Amounts of 0.1 to 0.2 ml of the DNA preparation were mixed with 0.05 ml of ^{14}C-labeled λ DNA and layered on 4.5 ml of a discontinuous sucrose gradient. The ^{14}C-labeled mature λ DNA was used as a marker in these experiments. The preparations were centrifuged in the SW-39 head of a Spinco model L for 2 hr at 38,000 rev/min. A discontinuous gradient of 5 to 25% sucrose in 0.01 M Tris-0.001 M ethylenediaminetetraacetic acid, pH 7.4, was used. The gradient was prepared by successively layering 1 ml of 20, 15, 10, and 5% sucrose solution on top of 0.5 ml of 25% sucrose solution. Fractions of 0.15 ml were collected from the gradient by syphon directly into counting vials. The samples were counted in a triton toluene scintillation as previously described (14).

RESULTS

Sedimentation of λ DNA. The synthesis of λ DNA after lysogenic induction was measured by the amount of incorporation of radioactive thymidine. This method has the disadvantage of not distinguishing between λ DNA synthesis and the synthesis of the host bacterial DNA. However, in these experiments, the ultraviolet light irradiation used (560 ergs/mm^2) was sufficient to depress the cellular synthesis of host DNA, while permitting the normal synthesis of phage DNA. This is illustrated in Table 1, in which DNA synthesis in an ultraviolet light-induced, wild-type lysogen is compared with that of the irradiated nonlysogenic, isogenic control strain and some induced defective lysogens. It has previously been shown

(14) that, under the inducing conditions used here, λ DNA synthesis starts about 40 min after ultraviolet irradiation of *E. coli* W3350(λ). The incorporation of ^{3}H-thymidine into DNA by the nonlysogenic cell, W3350 (S), was low (Table 1). At 45 min after irradiation, incorporation was 15%, and at 60 and 70 min there was less than 6% of the incorporation by the wild-type lysogen, W3350(λ). The defective λ mutants sus O and P may synthesize slightly more DNA at 45 min than the nonlysogen, but by 60 and 70 min after induction, DNA synthesis by the nonlysogen and λ sus O and P are similar. On the other hand, the defective mutant λ sus N, at 60 min after induction, incorporates significantly more ^{3}H-thymidine into DNA than does the nonlysogen. In fact, the amount of DNA synthesized by λ sus N at this time period is about 36% of that synthesized by the wild-type lysogen.

It should also be noted that, in contrast to the normal phage DNA synthesis, the pulse-labeling experiments shown in Table 1 indicate that less phage DNA is synthesized by the λ sus Q mutant at 70 min after induction than at 60 min after induction. This is the opposite of the pattern of DNA synthesis which occurs after induction of the wild-type lysogen, W3350(λ). The synthesis of DNA by λ sus Q at 45 and 60 min after induction, in the experimental conditions employed here, was about 70% of that synthesized by the normal λ phage at the same time periods.

The sedimentation characteristics of the pulse-labeled DNA synthesized by the wild-type lysogen and the nonlysogenic cell were also compared. The DNA synthesized by W3350(S) at 45, 60, or 70 min after irradiation (Fig. 1) was mainly of a low molecular weight which stays on top of the gradient. As previously reported (14), when the lysogenic cell is pulse labeled 45 min after induction, most of the newly synthesized DNA migrates in a sucrose gradient at about the same rate as the mature λ DNA marker (Fig. 1A).

TABLE 1. *Incorporation of ^{3}H-thymidine into DNA*

Time of addition of ^{3}H-thymidine after irradiation[a]	^{3}H-thymidine incorporated/10^8 cells (μμmoles)					
	W 3350(λ)	W3350(S)	W3350(λ) susN$_7$	W3350(λ) susO$_8$	W3350(λ) susP$_3$	W3350(λ) susQ$_{21}$
min						
45	2.2	0.34	0.56	0.95	0.88	1.5
60	8.0	0.48	2.2	0.56	0.76	6.0
70	24.0	0.90	2.1	0.96	0.78	2.6

[a] Bacterial cultures in logarithmic growth were irradiated with ultraviolet light. After reincubation for 45, 60, or 70 min, the cultures were pulse-labeled for 1 min with ^{3}H-thymidine (final concentration, 6 μc/ml, 0.7 mμmoles/ml). Thymidine (final concentration, 2 μmoles/ml) was then added. The cultures were incubated for an additional 5 min and then cooled; the DNA was extracted as described in Materials and Methods.

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The early form of λ DNA, migrating at the same rate as the mature λ DNA marker, is called Intermediate I (17). By 60 min after induction, the synthesized, pulse-labeled DNA sediments broadly, showing two peaks with some intermediary material. One peak (fraction 18) is similar in sedimentation properties to the linear λ DNA, and the second peak (fraction 11) migrates about 1.7 times faster than the marker phage DNA (Fig. 1B). This faster-sedimenting DNA is referred to as concatenated DNA and its properties have been discussed elsewhere (14). By 70 min after induction, the fast-sedimenting pulse-labeled DNA species is no longer detectable. The DNA sediments primarily in the region of the linear, mature DNA and as a poorly defined band moving 1.3 to 1.4 times faster than the mature λ DNA.

Synthesis of DNA by defective λ phages. When *E. coli* W3350, lysogenic for the early defective mutants of λ, sus N, sus O, sus P, and T₁₁, were induced by ultraviolet light, it was found that the synthesis of DNA was markedly reduced from that found with lysogenic strains carrying a normal prophage. Cultures of these defective lysogens were pulse-labeled with ³H-thymidine at 45, 60, and 70 min after induction. The DNA was extracted from the cells and placed on a neutral sucrose gradient as described in Materials and Methods. The sedimentation characterisics of the pulse-labeled DNA obtained from the defective mutants was found to vary. DNA extracted from λ sus O and sus P presented a sedimentation profile similar to that of the nonlysogenic irradiated culture (Fig. 1). Most of the radioactivity incorporated into the DNA was present as a slow-sedimenting material remaining near the top of the sucrose gradient with a small peak, probably bacterial DNA, at fraction 12 of the gradient.

After induction of *E. coli* C600, lysogenic for the defective phage λ T₁₁, the sucrose gradient sedimentation profiles of the pulse-labeled DNA obtained at 45, 60, and 70 min were indistinguishable from those obtained with the nonlysogenic cell of strain W3350 (Fig. 1).

The pulse-labeled DNA synthesized by *E. coli* W3350, lysogenic for λ sus N₇ at 45, 60, and 70 min after induction, is shown in the sedimentation profiles of Fig. 2. At 45 min after induction (Fig. 2A), the pulse-labeled DNA sediments in a peak migrating at about the same rate as the linear λ DNA marker. By 60 min after induction (Fig. 2B), the DNA synthesized sediments in a sucrose gradient as a band containing two discrete peaks. One peak (fraction 17) migrates 1.3 to 1.4 times faster than both the second peak (fraction 20) and the marker λ DNA. The DNA pulse-labeled at 70 min after induction (Fig. 2C)

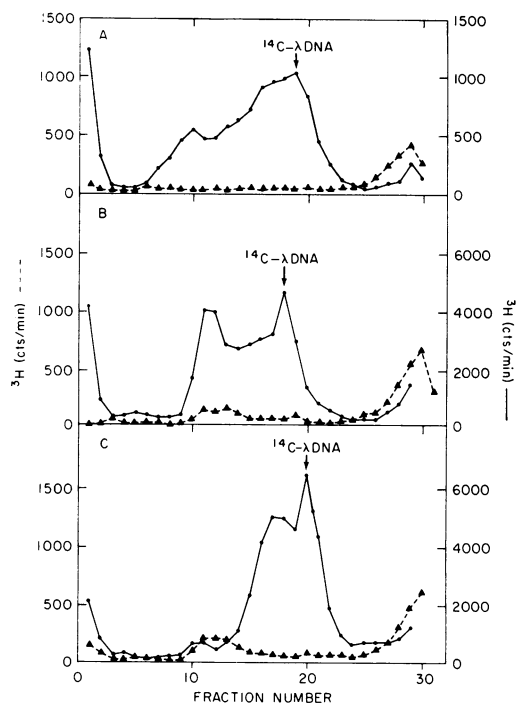FIG. 1. *Sedimentation of pulse-labeled Escherichia coli strains W3350(S) and W3350(λ) DNA obtained at various times after induction. E. coli W3350(S) (▲) and W3350(λ) (●) were induced separately and ³H-thymidine (final concentration, 6 μc/ml) was added at 45 (A), 60 (B), or 70 (C) min after induction. After a 1-min exposure to the ³H-thymidine, nonlabeled thymidine (final concentration, 0.06 μmole/ml) was added for an additional 5 min. The cells were then collected by centrifugation and the DNA was extracted and sedimented in 4.5 ml of a 5 to 25% sucrose gradient, pH 7.4, for 120 min at 38,000 rev/min. ¹⁴C-labeled λ DNA was added to the top of each gradient before centrifugation. Sedimentation in the gradient is from right to left, with fraction 1 representing the bottom of the tube.*

is present both as a form (fraction 21) migrating with the mature λ DNA and as material sedimenting about 1.4 times faster. A poorly defined intermediary band is also present.

The sedimentation profiles of the DNA synthesized by *E. coli* strain W3350, lysogenic for λ sus Q, pulse-labeled at various times after ultraviolet-light induction, are seen in Fig. 3. At 45 min after induction (Fig. 3A), the labeled DNA migrates primarily with the linear DNA marker. At 60 min (Fig. 3B) and 70 min (Fig. 3C) after induction, the DNA migrates in a broad band with two peaks. One peak (fraction 21) migrates with the linear λ DNA marker, and the other peak (fraction 15) migrates approximately 1.5 times faster than the linear λ DNA.

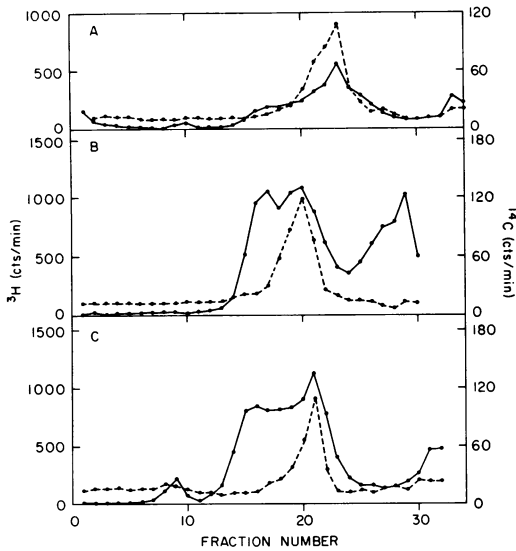FIG. 2. *Sedimentation of λ DNA from pulse-labeled Escherichia coli strain W3350(λ sus N_7) lysed at various times after induction. E. coli W3350(λ sus N_7) was induced and pulse-labeled with ³H-thymidine as described in Fig. 1. (A)45 min, (B)60 min, (C)70 min. ³H(●, solid line); ¹⁴C λ DNA marker (●, dashed line).*
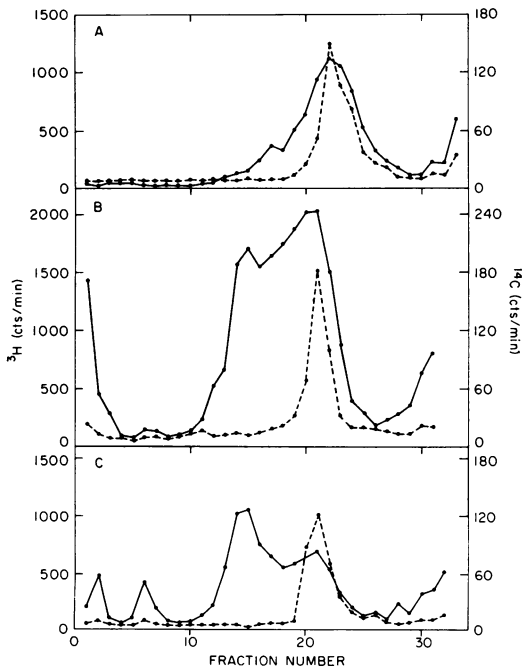

FIG. 3. *Sedimentation of DNA from pulse-labeled Escherichia coli strain W3350(λ sus Q_{21}) lysed at various times after induction. E. coli W3350(λ sus Q_{21}) was induced and pulse-labeled with ³H-thymidine as described in Fig. 1. (A)45 min, (B)60 min, (C)70 min. ³H (●, solid line); ¹⁴C λ DNA marker (●, dashed line).*

DISCUSSION

The early mutants of λ are defective to varying degrees in their ability to synthesize phage nucleic acids and proteins. The λ mutant sus N is extremely defective and has been reported not to synthesize detectable amounts of the phage enzymes, exonuclease (12) or lysozyme (11), mRNA (9), or any mature phage particles (9). Genetic experiments (2) and experiments involving specific hybridization on agar DNA columns (9) have also indicated that the λ mutant sus N produces little if any phage DNA. Eisen et al. (6), using *E. coli* strain Y_{10} lysogenic for early defective mutant T_{27} (which has a mutation in the N cistron), pulse-labeled the DNA with radioactive thymidine after mitomycin induction. They found that the amount of radiosotope incorporated into DNA by the T_{27} lysogen, although small, was significantly higher than that incorporated by a cured lysogen. In agreement with the latter work, our experiments indicate that after ultraviolet light induction the defective sus N lysogen did incorporate measurably more ³H-thymidine into DNA than did an irradiated nonlysogenic, isogenic cell or the other early mutants sus O, sus P, and T_{11}. The DNA synthesized by λ sus N, when applied to a neutral sucrose gradient, gave a sedimentation profile which had peaks similar to those found after normal phage induction. At 45 min after induction, the DNA synthesized by λ sus N (Fig. 2A) had the sedimentation characteristics of mature λ DNA and the Intermediate I found after normal phage infection (Fig. 1A; 17). At 60 min after induction, the sus N mutant of λ synthesizes DNA sedimenting with the DNA marker plus an additional form migrating 1.3 to 1.4 times faster than the marker (Fig. 2B). Unlike the normal phage DNA profile at this time, the N mutant does not appear to synthesize the phage DNA form which migrates approximately 1.7 times faster than marker λ DNA (Fig. 1B). Phage λ sus N still continues to synthesize the two DNA species migrating with marker λ DNA and 1.4 times faster, as well as some ill-defined intermediate heterogeneous DNA at 70 min after induction.

The faster-sedimenting DNA species synthesized by the λ sus N phage at 60 min after induction was isolated from a sucrose gradient and partially characterized. The sedimentation of this intermediate form is not due to simple aggregation of the DNA molecule. Heating at 75 C in 0.6 M NaCl at pH 7.4, followed by rapid cooling, which destroys known aggregates of λ DNA (8), did not affect the sedimentation of this intermediate. The intermediate is also probably not a circular duplex molecule, since its relative sedi-

mentation properties at pH 7.4 and 12.2 in sucrose gradients are not those of known covalently linked circular λ DNA (1). Previous experiments have also shown that W3350 λ sus N does not synthesize a twisted circular DNA species after lysogenic induction (13).

Hybridization experiments using the DNA-agar technique (4) confirmed that both peaks of the pulse-labeled DNA synthesized by λ sus N at 60 min after induction are forms of λ DNA. The DNA which sediments with mature λ DNA hybridized 44% with mature λ DNA imbedded in agar. The DNA peak sedimenting about 1.4 times faster than mature λ DNA hybridized 40% with the DNA imbedded in agar. Furthermore, the thermal elution pattern obtained after such hybridization is similar to that found with whole λ DNA itself.

The other early mutants of λ, sus O, sus P, and T_{11}, were also investigated for their ability to synthesize the intermediate DNA species. As previously reported, genetic (2), DNA hybridization (9), and pulse-labeling (6) experiments have all indicated that these mutants synthesize no detectable phage DNA. In the experiments reported in this paper, it was not possible to demonstrate the synthesis of any phage DNA intermediates by any of these mutants. Although unable to synthesize detectable phage DNA, the λ sus mutants O and P synthesize both the phage exonuclease and lysozyme (11). T_{11}, on the other hand, has been reported to produce a greatly elevated amount of exonuclease (12) and no detectable lysozyme (11). The apparent inability to synthesize any phage DNA, while still retaining the ability to synthesize some phage-specific enzymes and a limited amount of mRNA (9), suggests the possibility that the prophage itself is transcribed.

The mutant of λ sus Q has previously been shown by hybridization experiments (9) to synthesize approximately 75% of the DNA synthesized during normal phage development. The mutant is able to synthesize reduced amounts of mRNA (9) and lysozyme (11) and to synthesize exonuclease (11) and circular λ DNA (13). In the experiments reported here, λ sus Q incorporated about 70% as much ^{3}H-thymidine into DNA as the normal phage at both 45 and 60 min after induction. The decrease in radioisotope incorporation into the DNA observed 70 min after induction is unexplained. It could be due to decreased synthesis or increased degradation of the newly formed DNA molecule. At 45 min after induction (Fig. 3A), λ sus Q presents a sedimentation profile similar to the one seen in normal phage development (Fig. 1A), synthesizing primarily the early Intermediate I. By 60 min after

induction (Fig. 3C), λ sus Q also synthesizes a DNA molecule with faster sedimenting characteristics. Considerable variability in the sedimentation of this faster migrating molecule has been observed. The sedimentation was found to vary from approximately 1.3 to 1.5 times the linear marker DNA. The intermediate phage DNA shown in Fig. 3B, migrating approximately 1.5 times faster than the λ DNA marker, is not believed to be a circular or an aggregated molecule. When isolated from a sucrose gradient, it was found to maintain its fast sedimentation characteristics relative to mature λ DNA in an alkaline sucrose (1.25 ×) gradient or after heating at 75 C in 0.6 M salt at pH 7.4 (1.4 ×). Hydrodynamic shear studies, carried out as previously described (14), show that this fast-sedimenting DNA species is more sensitive to shearing forces than the mature phage DNA. At speeds of 1,400 rev/min, the fast-sedimenting DNA molecule synthesized by sus Q is sheared to a form which migrates in a neutral sucrose gradient with mature λ DNA marker. At speeds of 4,000 rev/min, the fast-sedimenting DNA species synthesized by λ sus Q and mature λ DNA are sheared and migrate more slowly than the linear phage DNA marker.

Hybridization experiments using the DNA agar technique (4) have shown that the fast-sedimenting DNA species synthesized by λ sus Q is a form of λ DNA. The fast-sedimenting DNA species showed essentially complete hybridization with wild-type DNA imbedded in agar. The thermal elution of the hybridized DNA gave a pattern similar to that found with whole λ DNA itself.

Because of the variation in sedimentation characteristics, it is not possible to state whether this fast-sedimenting DNA intermediate synthesized by sus Q is closely related to the longer DNA form synthesized during normal phage development. Further studies on the synthesis of DNA by the λ mutant sus Q could lead to an understanding of its proposed role in regulating the synthesis of late phage functions (5).

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