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Initiation of DNA replication on single-stranded DNA templates catalyzed by purified replication proteins of bacteriophage λ and *Escherichia coli*

(O and P initiators/dnaB protein/dnaJ and dnaK heat shock proteins/prepriming events/primosome)

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ABSTRACT **Initiation of bacteriophage λ DNA replication at the chromosomal origin depends on the λ O and P replication proteins. These two viral initiators, together with an *Escherichia coli* protein fraction, promote the replication *in vitro* of single-stranded circular DNA chromosomes such as that of bacteriophage M13. This nonspecific strand initiation reaction, which we have termed the "λ single-strand replication reaction," has now been established with eight purified proteins, each of which is also required for replication of the phage λ chromosome *in vivo*. An early rate-limiting step in the overall reaction is the ATP-dependent assembly of an activated nucleoprotein prepriming complex. In this step the λ O and P initiators cooperate with the *E. coli* dnaJ and dnaK proteins to transfer the bacterial dnaB protein onto M13 DNA that is coated with the single-stranded DNA-binding protein. Multiple RNA primers are synthesized on each DNA circle when isolated prepriming complex is incubated with primase and rNTPs. In the complete system, DNA polymerase III holoenzyme extends the first primer synthesized into full-length complementary strands. Because the properties of this system are closely analogous to those found for the replication of φX174 viral DNA by *E. coli* proteins, we infer that a mobile prepriming or priming complex (primosome) operates in the λ single-strand replication reaction.**

Replication of the bacteriophage λ chromosome in *Escherichia coli* is a complex process requiring the concerted action of more than 20 different polypeptides. As with most temperate phages, λ relies heavily on the host cell for replication machinery. Only two of the required polypeptides, the λ O and P replication initiators, are encoded by the viral chromosome. These two phage proteins are believed to direct the host replication machinery to the λ replication origin (*ori*λ), from which replication forks are propagated bidirectionally (see refs. 1 and 2 for recent reviews of λ replication). The sequence of events by which the λ O and P proteins accomplish this task has not been determined, although clues have been provided by genetic and biochemical studies which document interactions of the λ O protein with *ori*λ (3–5) and of the λ P protein with the λ O protein (6, 7) and with the host dnaB, dnaJ, and dnaK proteins (8–14).

Direct biochemical analysis of the molecular mechanisms involved in the initiation of phage λ DNA replication is now possible. *In vitro* systems that support the specific replication of superhelical plasmids carrying *ori*λ have been developed in several laboratories (15–17). Recently, we described a related soluble enzyme system in which the λ O and P proteins and a partially purified *E. coli* protein fraction catalyze initiation of nascent DNA chains on single-stranded (ss) DNA coated with the ss DNA-binding protein (SSB) (18). Strand initiation in this system, which we termed the "λ single-strand (SS) replication reaction," is nonspecific with regard to template DNA sequence—i.e., the presence of *ori*λ is unnecessary. However, several host proteins that are known to function in λ DNA replication *in vivo* participate in this *in vitro* strand initiation reaction (18). We have proceeded to analyze the λ SS replication reaction in greater detail due to its relative simplicity and because it may share several of the enzymatic steps required for initiation of bidirectional DNA replication at *ori*λ. We now report that the λ SS replication reaction can be established with the λ O and P initiators and six *E. coli* replication proteins. Reconstitution of this replication system with purified proteins has enabled us to separate the overall initiation reaction into prepriming, priming, and DNA synthesis steps.

MATERIALS AND METHODS

Reagents and Materials. Sources were as follows: proteinase K, EM Laboratories (Elmsford, NY); Hepes, Research Organics (Cleveland, OH); adenosine 5′-[γ-thio]triphosphate) (ATP[γS]) and 5′-adenylyl imidodiphosphate (AMP-*P*[NH]*P*), Boehringer Mannheim; [5-^{3}H]CTP (10–25 Ci/mmol), [8-^{3}H]GTP (10–25 Ci/mmol), and [5,6-^{3}H]UTP (25–40 Ci/mmol), ICN; [α-^{32}P]dNTPs and [α-^{32}P]-rNTPs (each at 400–450 Ci/mmol) and [γ-^{32}P]ATP (3000 Ci/mmol), Amersham (1 Ci = 37 GBq); Bio-Gel A-15m agarose (100–200 mesh), Bio-Rad. The sources of all other reagents and materials have been described previously (4, 16, 18).

Phage Strains. M13mp8 phage (19) were obtained from Bethesda Research Laboratories. M13G*ori*101 phage (20) were obtained from D. Ray.

Enzymes. Highly purified λ and *E. coli* replication proteins were as follows: λ O protein (fraction III, 8×10^4 units/mg) (4); λ P protein (fraction V, 5.8×10^5 units/mg) (11, 21); SSB (fraction III, 4×10^4 units/mg) (unpublished data); dnaB protein (fraction III, 4×10^5 units/mg) (ref. 22; unpublished data); primase (fraction IV, 9.7×10^5 units/mg) (ref. 23; unpublished data); DNA polymerase III holoenzyme (fraction V, 5.7×10^5 units/mg) (24); dnaJ protein (fraction V, 9.8×10^4 units/mg) (25); dnaK protein (fraction VII, 9×10^3 units/mg) (26). All replication proteins were greater than 90% pure. A unit of activity catalyzes the incorporation of 1 pmol of deoxynucleotide per min into acid-insoluble material under standard assay conditions.

Assay of DNA Replication. The replication conditions were a modification of those reported previously (18). The stan-

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Abbreviations: ss DNA, single-stranded DNA; SSB, single-stranded DNA-binding protein; λ SS replication reaction, λ single-strand replication reaction; ATP[γS], adenosine 5′-[γ-thio]triphosphate; AMP-*P*[NH]*P*, 5′-adenylyl imidodiphosphate.

dard λ SS replication reaction mixture, 25 μl, contained the following: Hepes/KOH at pH 7.6, 40 mM; magnesium acetate, 11 mM; ATP, 2 mM; CTP, GTP, and UTP, each 500 μM; dATP, dCTP, and dGTP, each 192 μM; dTTP, 72 μM with [*methyl*-^{3}H]dTTP at 100–125 cpm/pmol of total deoxynucleotide; rifampicin, 20 μg/ml; bovine serum albumin, 50 μg/ml; M13mp8 DNA, 150 ng (450 pmol deoxynucleotide equivalent); SSB, 1.9 μg; λ O protein, 150 ng; λ P protein, 45 ng; dnaB protein, 80 ng; dnaJ protein, 150 ng; dnaK protein, 4 μg; primase, 85 ng; and DNA polymerase III holoenzyme, 140 ng. For replication from the phage G4 complementary strand origin, the standard reaction mixture was similar to that described above, except that it contained M13G*ori*101 DNA (450 pmol) and just three proteins: SSB (1.9 μg), primase (85 ng), and DNA polymerase III holoenzyme (140 ng). Unless noted otherwise, reaction mixtures were assembled at 0°C and DNA synthesis was measured (16) after a 20-min incubation at 30°C.

RNA Primer Synthesis. The standard λ priming reaction, 25 μl, contained the following: Hepes/KOH, pH 7.6, 40 mM; magnesium acetate, 11 mM; bovine serum albumin, 50 μg/ml; rifampicin, 20 μg/ml; ATP, 2 mM; CTP, GTP, and UTP, each 50 μM; [^{3}H]CTP, [^{3}H]GTP, and [^{3}H]UTP, each at 1000–2000 cpm/pmol; M13mp8 DNA, 450 pmol; SSB, 1.9 μg; λ O protein, 150 ng; λ P protein, 45 ng; dnaB protein, 80 ng; dnaJ protein, 150 ng; dnaK protein, 4 μg; and primase, 85 ng. For primer synthesis at the phage G4 complementary strand origin, the template was M13G*ori*101 DNA (450 pmol), and the only proteins added to the reaction mixture were SSB (1.9 μg) and primase (85 ng). Reaction mixtures were assembled at 0°C. After a 20-min incubation at 30°C, an aliquot of the reaction mixture was spotted on Whatman DE 81 DEAE-cellulose discs and treated as previously described (27).

Gel Electrophoresis. Polyacrylamide gel electrophoresis in 7 M urea was performed as previously described (28).

RESULTS

Reconstitution of the λ SS Replication Reaction with Purified Proteins. We previously reported the development of a soluble enzyme system in which the phage λ O and P initiator proteins, acting in concert with an *E. coli* protein fraction, promoted the replication of SSB-coated, single-stranded circular DNAs such as M13mp8 (18). We found that we could reconstitute this λ SS replication system with a mixture of eight highly purified λ and *E. coli* replication proteins (Table 1). Omission of the λ O or P replication proteins or any of six *E. coli* replication proteins from the system caused a reduction in DNA synthesis to less than 1/10th of that made in the complete system. The use of sufficient quantities of purified proteins eliminated any necessity for a hydrophilic polymer (such as polyvinyl alcohol) or for an ATP regenerating system, both of which stimulated replication in the crude system (18). When the λ SS replication reaction system was reconstituted with optimal levels of purified proteins, the complementary strand products were predominantly full-length (data not shown).

The level of *E. coli* SSB added to the purified protein system is critical. If the available template DNA is not completely coated with SSB, then dnaB protein and primase act via the general priming reaction to synthesize functional primers for DNA chain elongation (29). The general priming reaction can be completely suppressed by added SSB (Fig. 1) (29). In contrast, low levels of SSB stimulate the λ SS replication reaction (Fig. 1). Nevertheless, excessive levels of SSB cause inhibition of this reaction as well. All further experiments were performed at SSB levels (1.9 μg/450 pmol of ss DNA template) that completely suppress the general priming reaction (e.g., see squares, Fig. 2*A*) yet leave the λ SS replication pathway largely unimpaired.

Table 1. Requirements for the reconstitution of the λ SS replication reaction with purified proteins

Component omitted or added	DNA synthesis, pmol
None	263
−λ O protein	12
−λ P protein	13
−dnaB protein	12
−dnaJ protein	10
−dnaK protein	3
−Primase	2
−DNA polymerase III holoenzyme	1
−O, P, dnaJ, dnaK	14
−ATP	12
−CTP, GTP, and UTP	281
+Polyvinyl alcohol (5%, wt/vol)	215
+Creatine phosphate and kinase*	247

The standard λ SS replication reaction was carried out as described in *Materials and Methods*, except that individual components were omitted or added as indicated.

*Creatine phosphate and creatine kinase were added as previously described (18).

Formation of a Prepriming Intermediate in the λ SS Replication Reaction. As is the case for the replication of coliphage φX174 ss DNA (30–32), preincubation of template ss DNA with ATP and all required proteins, except primase and DNA polymerase III holoenzyme, enables an activated prepriming intermediate to be formed. Assembly of this prepriming intermediate in the λ SS replication reaction is indicated by a 10-fold enhancement of the initial rate of DNA synthesis (Fig. 2*A*) that occurs subsequent to the addition of primase and DNA polymerase III holoenzyme. Formation of the prepriming intermediate was complete after 10 min of incubation at 30°C (Fig. 2*B*). The similarity in the kinetics of formation of the prepriming intermediate (Fig. 2*B*) to the kinetics of DNA synthesis in the complete system in the absence of preincubation (Fig. 2*A*, open circles) suggests that

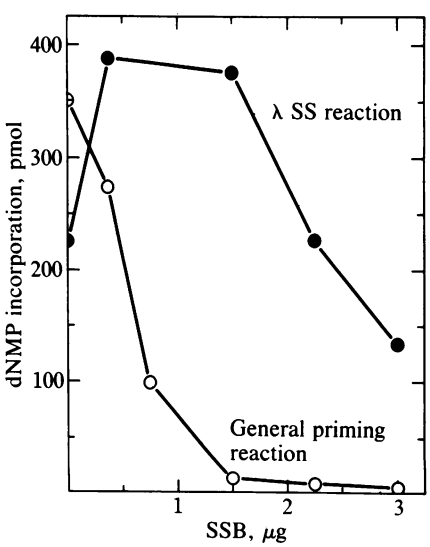

FIG. 1. Effect of SSB concentration on the initiation of complementary strand DNA synthesis on ss DNA templates. The λ SS replication reaction (●) was performed as described in *Materials and Methods*, except that the amount of SSB present was varied as indicated. The replication reaction using the *E. coli* general priming system (○) was performed in an identical fashion, but for these reactions the λ O and P proteins and the bacterial dnaJ and dnaK proteins were omitted from the system. For both reactions, SSB was added to the DNA template (M13mp8 DNA) prior to the addition of any other replication proteins.

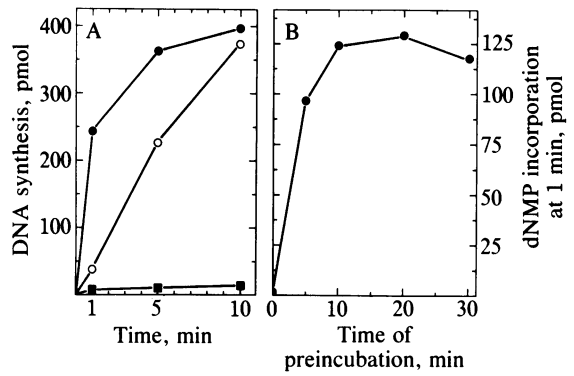

FIG. 2. Formation of a prepriming replication intermediate in the λ SS replication reaction. (*A*) Time course of DNA synthesis in the λ SS replication reaction before and after formation of the prepriming intermediate. The λ SS replication reaction was divided into two stages. The stage one reaction mixture (57 μl) contained the following: Hepes/KOH at pH 7.6, 70.2 mM; magnesium acetate, 19.3 mM; bovine serum albumin, 88 μg/ml; ATP, 3.5 mM; rifampicin, 35 μg/ml; SSB, 7.6 μg; M13mp8 DNA, 1.8 nmol; λ O protein, 600 ng; λ P protein, 180 ng; dnaB protein, 320 ng; dnaJ protein, 600 ng; dnaK protein, 16 μg. This mixture was either incubated at 30°C for 20 min (●) or kept at 0°C (○). Each first-stage mixture was supplemented with the stage two components required for priming and replication (100-μl final reaction volume): primase, 330 ng; DNA polymerase III holoenzyme, 330 units; and rNTPs and labeled dNTPs as described in *Materials and Methods*. Replication was initiated by incubation of the stage two mixture at 30°C. At the times indicated, 25-μl portions were removed and analyzed for DNA synthesis. A similar two-stage reaction was performed (with and without a first-stage incubation at 30°C) in which the λ O and P proteins and the *E. coli* dnaJ and dnaK proteins were omitted from both the first and the second stage incubations (■). (*B*) Time course of activation of M13mp8 DNA prior to priming. A series of stage one reaction mixtures was prepared as described above. Each mixture was placed at 30°C (zero time) and, at the times indicated, one of these mixtures was supplemented with stage two components (as described above) and incubated for 1 additional min at 30°C to permit rapid replication. A portion (25 μl) of each sample was analyzed for acid-insoluble radioactive material.

assembly of the prepriming intermediate is the rate-limiting step in the overall replication pathway. This conclusion is also supported by the fact that the initial rate of DNA synthesis is greatly elevated when the template DNA is first preincubated with the prepriming proteins.

Formation of active prepriming intermediate in the λ SS replication reaction requires, in addition to the ss DNA template, the λ O and P initiators and the *E. coli* SSB, dnaB, dnaJ, and dnaK replication proteins (Table 2). Moreover, hydrolysis of ATP, or another rNTP, is needed for assembly of the prepriming intermediate (Table 3). Nonhydrolyzable analogues of ATP, namely ATP[γS] and AMP-*P*[NH]*P*, do not support formation of the intermediate, and only dATP of the dNTPs is even weakly active in this regard. In contrast, assembly of the φX174 prepriming intermediate, the preprimosome, *is* supported by nonhydrolyzable ATP analogues (33, 34).

Isolation of a Prepriming Complex. The active intermediate that is formed prior to the priming step in the λ SS replication reaction can readily be isolated and freed of unassociated protein by filtration through agarose (Fig. 3). As estimated from the amount of starting template DNA that can be rapidly replicated after gel filtration, yields of the prepriming complex in these experiments varied from 17% to 50%. The isolated nucleoprotein intermediate represents a true prepriming complex, analogous to the φX174 preprimosome, as judged by the following criteria: (*i*) isolation of the complex depends upon prior incubation of the ss DNA template at 30°C with the required prepriming proteins and ATP (Fig. 3); (*ii*) DNA synthesis on the isolated intermediate requires the addition of primase as well as DNA polymerase III holoenzyme (no further addition of any of the prepriming proteins to the isolated complex is necessary) (Fig. 3); and (*iii*) DNA synthesis on the isolated intermediate is initiated and completed rapidly (within 3 min) (Fig. 4).

Table 2. Protein requirements for formation of an activated replication intermediate

Component omitted	Rapid DNA synthesis, pmol
None	163
M13mp8 DNA	4
λ O protein	5
λ P protein	5
dnaB protein	8
dnaJ protein	22
dnaK protein	9

A stage one preincubation mixture of 14 μl was prepared as described in the legend to Fig. 2*A*, except that individual components were omitted as indicated. After a 20-min preincubation at 30°C, each mixture was supplemented with the missing component, and with rNTPs, primase (85 ng), and DNA polymerase III holoenzyme (140 ng) and incubated for 1 min at 30°C. The amount of rapid DNA synthesis was determined.

Primer Synthesis in the λ SS Replication Reaction. We have examined the capacity of the reconstituted enzyme system to synthesize RNA primers for DNA chain elongation. When SSB-coated M13mp8 template DNA is incubated in the presence of rNTPs with the other five prepriming proteins (O, P, dnaB, dnaJ, and dnaK proteins) and primase, extensive RNA primer synthesis is obtained (200 ribonucleotide residues per input DNA circle) (Fig. 4). Little or no RNA synthesis (<5 residues per circle) is detected on SSB-coated DNA when any of the other six replication proteins are omitted from the priming mixture. After a 3–4 min lag, RNA primer synthesis proceeds linearly for approximately 40 min (Fig. 4). Not only is this lag greatly reduced when isolated prepriming intermediate is used as the substrate for primer synthesis but also the observed RNA synthesis ceases after 20–30 min (Fig. 4). When the isolated prepriming complex was used, approximately 125 ribonucleotide residues were incorporated per initial template circle (Fig. 4). In this experiment, however, only 20% of the starting template could be replicated to a duplex form after isolation of the inter-

Table 3. Nucleotide requirements for formation of an activated replication intermediate

Nucleotide present in preincubation	Rapid DNA synthesis, pmol
None	11
ATP	222
CTP	135
GTP	53
UTP	116
dATP	23
dCTP	3
dGTP	3
ATP[γS]	2
AMP-*P*[NH]*P*	5

A stage one preincubation mixture of 14 μl was prepared as described in the legend to Fig. 2*A*, except that the nucleotide present was varied as indicated. Both ATP[γS] and AMP-*P*[NH]*P* were present at 0.5 mM, whereas all other nucleotides were present at 2 mM. After a 20-min preincubation at 30°C, each mixture was supplemented with primase (85 ng), DNA polymerase III holoenzyme (140 ng), and dNTPs and all four rNTPs as described for the λ SS replication reaction. Rapid DNA synthesis was determined after a 1-min incubation at 30°C.

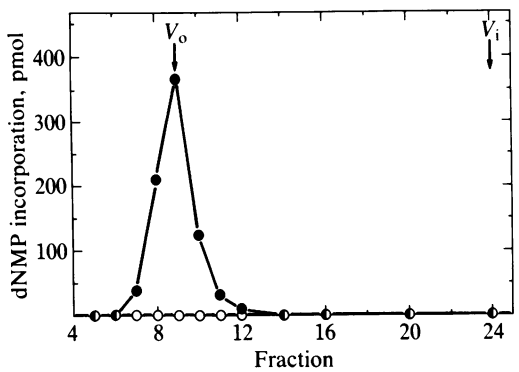

FIG. 3. Isolation by agarose gel filtration of an activated nucleoprotein prepriming complex formed in the λ SS replication reaction. A stage one reaction mixture of 57 μl was prepared (see Fig. 2 legend) and incubated for 20 min at 30°C. One-fourth of this mixture, containing 450 pmol of M13mp8 template DNA, was supplemented with stage two components, incubated for 1 min at 30°C, and analyzed for rapid DNA synthesis (263 pmol). The remainder of the stage one mixture was applied to a 1-ml Bio-Gel A-15m agarose column equilibrated at 4°C in 40 mM Hepes–KOH, pH 7.6/11 mM magnesium acetate/2 mM ATP and bovine serum albumin (11 μg/ml). One-drop fractions were collected. Each fraction was supplemented with stage two components (80 units each of primase and DNA polymerase III holoenzyme and rNTPs and labeled dNTPs as described in *Materials and Methods*), incubated 5 min at 30°C, and analyzed for DNA synthesis (●). ○, Data obtained from similar experiments in which either (*i*) the stage one mixture was not incubated at 30°C prior to gel filtration or (*ii*) primase was not added to the stage two incubation mixture. V_o, void volume; V_i, included volume.

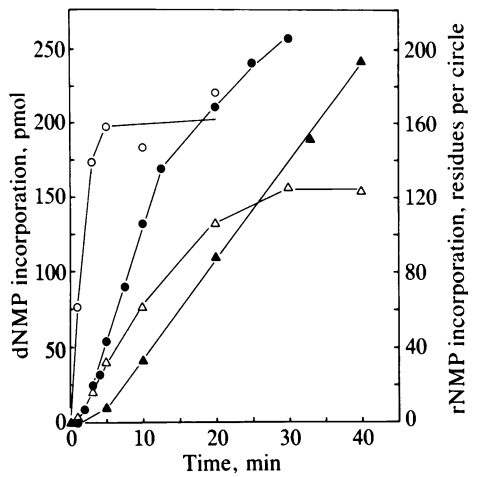

FIG. 4. Kinetics of RNA primer and DNA synthesis in the λ SS replication reaction. The time courses of DNA synthesis (●) and RNA primer synthesis (▲) in a one-stage reaction of 300 μl were determined as described in *Materials and Methods*, except that portions (25 μl) were removed at the indicated times for analysis. Also depicted are the time courses of DNA synthesis (○) and RNA primer synthesis (△) obtained when the activated nucleoprotein prepriming complex is isolated and used as the template. For these reactions, stage one reaction mixtures of 114 μl were incubated 20 min at 30°C and chromatographed over 1-ml Bio-Gel A-15m agarose columns as described in the legend to Fig. 2. The void volume material (150 μl), containing the nucleoprotein prepriming complex, was supplemented with stage two components: for DNA synthesis, primase (400 ng), DNA polymerase III holoenzyme (400 units), rNTPs and ^{3}H-labeled dNTPs (200 μl, final volume) were added; for RNA primer synthesis, primase (400 ng) and ^{3}H-labeled rNTPs (200 μl, final volume) were added. Both mixtures were incubated at 30°C and, at the indicated times, portions (40 μl for DNA synthesis, representing a maximum of 720 pmol of M13mp8 template; 20 μl for RNA primer synthesis) were removed for analysis.

mediate, presumably an indication that most SSB-coated circles lacked an active prepriming complex. Thus, it is likely that more than 600 ribonucleotide residues were incorporated per *active* prepriming complex.

Analysis by polyacrylamide gel electrophoresis indicated that the RNA primers synthesized in the λ SS replication reaction in the absence of DNA polymerase III holoenzyme vary in length from 15 to 60 residues, with an average length of approximately 30 nucleotides (Fig. 5, lanes E and F). Since primase initiates synthesis almost exclusively with ATP (28), it is also possible to obtain an estimate of the length of an average primer by measuring the ratio of [γ-^{32}P]ATP to [^{3}H]rNMP incorporated into RNA chains in a double-label experiment (see legend to Fig. 5). Such an analysis yields an average primer size of about 40 nucleotides. Thus, we calculate that 3–4 primer chains are synthesized per input circular DNA template and (correcting for recovery of active prepriming intermediate) that as many as 15–20 primers may be synthesized on each *active* DNA circle.

DISCUSSION

We have demonstrated that DNA strand initiation in the λ SS replication reaction can be catalyzed with a mixture of six *E. coli* replication proteins and the phage λ O and P initiators. The physiological relevance of this sequence-independent strand initiation reaction is strengthened by genetic studies that indicate that each of the eight proteins required *in vitro* is essential for replication of the λ chromosome *in vivo* (2).

Our analysis indicates that an early step in the λ SS replication reaction is the conversion of SSB-coated template DNA to an activated prepriming replication intermediate. In addition to SSB, the λ O and P proteins and the *E. coli* dnaB, dnaJ, and dnaK proteins are required for the ATP-dependent assembly of the nucleoprotein complex formed in this rate-limiting step. In experiments reported elsewhere (35), the activity of the isolated prepriming complex was found to be sensitive to antibody directed against the dnaB protein but resistant to antibodies directed against the λ O and P initiators or the dnaJ and dnaK proteins. We have not ruled out the possibility that one or more of these latter proteins is present in the prepriming complex in a form that is inaccessible to the antibodies directed against it. Thus far, however, the available data suggest that only dnaB protein and, perhaps, SSB are *functional* constituents of the activated prepriming complex.

The properties of the λ SS replication reaction described here are remarkably similar to those reported for the replication of φX174 viral DNA to the duplex form by *E. coli* proteins (28, 30–33). In both reactions the rate-limiting step is the ATP-dependent transfer of dnaB protein onto SSB-coated DNA to create an activated prepriming complex. In both systems dnaB protein is apparently recognized by primase as a locus for synthesis of multiple primers. In the case of φX174, synthesis of primer RNA transcripts is performed by the primosome, a multiprotein priming machine that migrates processively in the 5′-to-3′ direction along the template strand (33). In addition to dnaB protein and primase, the primosome is believed to contain at least three other *E. coli* proteins that participate in its formation—dnaC protein and proteins n and n′ (36, 37). Movement of the primosome is believed to be promoted by protein n′ (34). We surmise that the priming (or prepriming) complex that functions in the λ SS replication reaction, although apparently devoid of n′ protein, is also capable of translocation on the template strand, judging from the capacity of the complex to serve as a locus for the synthesis of multiple primers on each SSB-coated circle. If so, then the dnaB protein itself is likely to catalyze the movement.

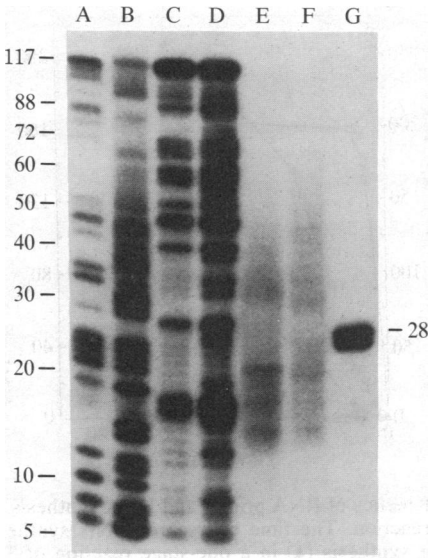

FIG. 5. Polyacrylamide gel electrophoresis of RNA primers synthesized in the λ SS replication reaction. Two separate stage one reaction mixtures (each 114 μl) were incubated 20 min at 30°C, and activated prepriming nucleoprotein complex was isolated by filtration through agarose as described (Fig. 2 legend), except that for one mixture 2 mM CTP replaced ATP in the column equilibration buffer. The nucleoprotein complex present in ATP (150 μl) was supplemented with primase (80 ng) and α-^{32}P-labeled CTP, GTP, and UTP (40 μM, 3000 cpm/pmol). The complex present in CTP was supplemented with primase (80 ng), ^{3}H-labeled GTP and UTP (40 μM, 1200 cpm/pmol), and [γ-^{32}P]ATP (20 μM, 63,500 cpm/pmol). Both mixtures were incubated 20 min at 30°C. Labeled primers annealed with the M13mp8 template were isolated by filtration of the mixtures through Bio-Gel A-15m columns equilibrated in 40 mM Hepes/KOH, pH 7.6. Typically, 70% of the RNA primer label applied to the column was recovered in the void volume fractions. In a control experiment the unique G4 complementary strand origin primer was synthesized as described in *Materials and Methods* by incubating M13G*ori*101 DNA (4500 pmol) with SSB (19 μg) and primase (400 ng) for 20 min at 30°C in the presence of α-^{32}P-labeled rNTPs (8000 cpm/pmol). A 3'-end-labeled, 117-base-pair fragment containing the *lacP–O* region was subjected to standard Maxam–Gilbert chemical degradation procedures by Paul Caron of this department and used for size standards. All samples were electrophoresed through an 8–20% gradient polyacrylamide gel containing 7 M urea. Lanes A–D, the G, G+A, C, and C+T chemical degradation reactions on the *lacP–O* fragment; lanes E and F, [γ-^{32}P]ATP-labeled and [α-^{32}P]NTP-labeled RNA primers made in the λ SS replication system; and lane G, the primer synthesized by primase at the phage G4 complementary strand origin.

Two *E. coli* heat shock proteins, the bacterial *dnaJ* and *dnaK* gene products, participate in λ DNA replication *in vivo* and *in vitro* (11–14, 18, 38, 39). The dnaJ and dnaK proteins functionally interact with the λ P replication protein (11–14), which, in turn, forms a tight complex with the *E. coli* dnaB protein, thereby suppressing the known enzymatic activities of the latter protein (9–11, 21). Moreover, as demonstrated here, the dnaJ and dnaK proteins participate in the transfer of dnaB protein into the activated nucleoprotein prepriming complex that is formed in the λ SS replication reaction. Additional studies (unpublished data) indicate that (*i*) a P protein–dnaB protein complex interacts with λ O protein bound at *ori*λ and (*ii*) the dnaJ and dnaK proteins act after dnaB protein and the λ O and P proteins in the initiation of λ*dv* plasmid DNA replication *in vitro*. It is possible that the dnaJ and dnaK proteins function to reactivate dnaB protein (suppressed by λ P protein in a nonfunctional prepriming complex).

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