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Studies on the initiation and elongation reactions in the simian virus 40 DNA replication system

(DNA polymerase α–DNA primase complex/oligoribonucleotide primer/leading-strand synthesis/protein–DNA complexes)

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ABSTRACT **The synthesis of oligoribonucleotides by DNA primase in the presence of duplex DNA containing the simian virus 40 (SV40) origin of replication was examined. Small RNA chains (10–15 nucleotides) were synthesized in the presence of the four common ribonucleoside triphosphates, SV40 large tumor antigen (T antigen), the human DNA polymerase α (pol α)–DNA primase complex, the human single-stranded DNA-binding protein (HSSB), and topoisomerase I isolated from HeLa cells. The DNA primase-catalyzed reaction showed an absolute requirement for T antigen, HSSB, and pol α. The requirement for HSSB was not satisfied by other SSBs that can support the T-antigen-catalyzed unwinding of DNA containing the SV40 origin of replication. Oligoribonucleotide synthesis occurred with a lag that paralleled the lag observed in DNA synthesis. These results indicate that the specificity for the HSSB in the SV40 replication reaction is due to the pol α–primase-mediated synthesis of the Okazaki fragments. In contrast to this specificity, the elongation of Okazaki fragments can be catalyzed by a variety of different DNA polymerases, including high levels of pol α, the polymerase δ holoenzyme, T4 polymerase holoenzyme, the *Escherichia coli* polymerase III holoenzyme, and other polymerases. These observations suggest that leading-strand synthesis in the *in vitro* SV40 replication system can be nonspecific.**

The replication of DNA containing the simian virus 40 (SV40) origin has been divided into a number of discrete steps (1–3). These include (*a*) the ATP-dependent binding of SV40 large tumor antigen (T antigen) at the core origin and the formation of hexamers on the DNA, followed by multiple changes at the origin that activate the intrinsic T-antigen DNA helicase. This results in (*b*) the ATP-dependent unwinding reaction, which requires a topoisomerase that relieves positive superhelicity generated by the movement of the T antigen through the duplex and a single-stranded DNA-binding protein (SSB) that binds the single strands generated by the displacement of the duplex. Prior to extensive unwinding, T antigen binds the DNA polymerase (pol) α–primase complex and facilitates (*c*) the initiation of small RNA primers by primase, which, coupled to (*d*) the action of pol α, results in the formation of small Okazaki fragments. Further unwinding leads to the continued generation of short DNA chains on the lagging strands. DNA primers can then be used for (*e*) the synthesis of the leading strand, which involves the addition of activator 1 (A1), also called RF-C (4), and proliferating-cell nuclear antigen (PCNA) to the 3′-hydroxyl end of the primer–template, permitting elongation by pol δ (4, 5). DNA synthesis continues until chains are juxtaposed; then oligoribonucleotides are removed by the combined action of RNase H and a 5′ → 3′ exonuclease, and gaps are filled in by a DNA polymerase and closed by DNA ligase. The semiconserved DNA products are resolved from one another by topoisomerase II, resulting in two circular closed duplex DNA molecules.

We have examined the requirements for step *c*, the synthesis of oligoribonucleotides, which was measured in the absence of DNA synthesis. We found that the synthesis of small oligoribonucleotides in the SV40 replication system required T antigen, pol α, primase, and the human SSB (HSSB). Reaction mixtures containing only primase, T antigen, and HSSB did not support primer synthesis. Synthesis was largely restored by the addition of pol α.

We have also examined step *e*, in which pol δ and its auxiliary proteins, A1 and PCNA, catalyze extensive DNA elongation. We learned that a variety of DNA polymerases could replace the pol δ holoenzyme. The polymerases that can elongate low levels of primers worked effectively in place of pol δ. These included the *Escherichia coli* pol III holoenzyme as well as the T4 pol holoenzyme system. Previous studies have shown that high levels of pol α can also support extensive synthesis of long DNA chains (6). The replication of SV40 DNA by these different DNA polymerases, however, specifically required the addition of T antigen, the pol α–primase complex, and HSSB. Tsurimoto *et al.* (7) reported similar results with the T4 holoenzyme used in place of the pol δ holoenzyme.

These results indicate that the interactions between HSSB, T antigen, and the pol α–primase complex on the SV40 origin-containing (ori$^+$) DNA are highly specific. In contrast, the elongation of primers, resulting in the accumulation of long DNA chains arising from the leading-strand template, appears to be nonspecific.

MATERIALS AND METHODS

Preparation of Replication Proteins. The following proteins were prepared as previously described: pol α–primase complex and topoisomerase I (6); PCNA (7); adenovirus DNA-binding protein (AdDBP) (8); HSSB (9); T antigen from baculovirus-infected insect cells, A1, and PCNA-dependent pol δ (10). DNA polymerases and SSBs obtained commercially included phage T4 pol (New England Biolabs), T7 pol (Sequenase, United States Biochemical), *Thermus aquaticus* (*Taq*) pol (IBI), *E. coli* pol I Klenow fragment (Boehringer Mannheim), T4 gene *32* protein (gp32, a SSB; Pharmacia LKB), and *E. coli* SSB (United States Biochemical). T4 gp44/62 and gp45 were generously provided by N. Nossal

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Abbreviations: SV40, simian virus 40; SSB, single-stranded DNA-binding protein; HSSB, human SSB; PCNA, proliferating-cell nuclear antigen; A1, activator 1; pol, specified DNA polymerase; SV40 ori$^+$ DNA, DNA containing the SV40 origin of replication; ss(c), single-stranded (circular); T antigen, SV40 large tumor antigen; nt, nucleotide(s); AdDBP, adenovirus DNA-binding protein (adenovirus-encoded SSB); T4 gp32, protein encoded by gene *32* of bacteriophage T4 (other gene products of phage T4 or T7 are abbreviated similarly).

(National Institutes of Health). *E. coli* pol III* and the β subunit (*dnaN* gene product) were prepared as described (11) or obtained from K. Marians (Sloan-Kettering Institute) and M. O'Donnell (Cornell University School of Medicine). T7 gp2.5, a SSB, was generously provided by C. C. Richardson (Harvard Medical School).

pol α–Primase Assay and Separation. pol α and primase were purified and measured as described (6). One unit of pol α incorporated 1 nmol of dTMP into DNA after 30 min at 30°C; one unit of primase activity supported the incorporation of 1 nmol of dAMP in the presence of $(dT)_{350}$, 5 mM ATP, and 0.5 unit of *E. coli* pol I (Klenow fragment) after 30 min of 30°C, as described (12). The separation of pol α and primase was carried out as reported (12). In the assay for primase activity, pol α–primase complex scored 3 times higher than primase alone.

SV40 *in Vitro* Replication System. Replication activity was assayed in reaction mixtures (30 μl) containing 40 mM creatine phosphate (di-Tris salt, pH 7.7); 7 mM $MgCl_2$; 0.5 mM dithiothreitol; 4 mM ATP, 200 μM UTP, GTP, and CTP; 100 μM dATP, dGTP, and dTTP; 20 μM [α-^{32}P]dCTP (20 cpm/fmol); 1 μg of creatine kinase; 0.22 μg of circular pSVLD ori$^+$ DNA [10 kilobases (kb) in size (13)]; 10 μg of bovine serum albumin; 0.9 μg of T antigen, 0.5–1 μg of HSSB, 1200 units of topoisomerase I, and each polymerase and other components as indicated. Mixtures were incubated as indicated at 37°C and aliquots were used to determine the amount of acid-insoluble radioactivity. When products were analyzed by gel electrophoresis, reactions were terminated by the addition of 20 mM EDTA, 1% SDS, and 40 μg of tRNA. Proteinase K (0.1 mg/ml) was added and the mixture was incubated for 30 min at 37°C. After phenol/chloroform extraction, products were precipitated with ethanol twice. Electrophoresis (3 V/cm, 12 hr) was carried out using 1.0 or 1.2% agarose gels containing 30 mM NaOH and 1 mM EDTA. After electrophoresis, gels were dried and autoradiographed.

Measurement of Oligoribonucleotide Synthesis in the Monopolymerase System. The complete system (40 μl) contained 40 mM creatine phosphate (di-Tris, pH 7.7), 7 mM $MgCl_2$, 0.5 mM dithiothreitol, 4 mM ATP, 1 μg of creatine kinase, 10 units of human placental RNase inhibitor, 200 μM CTP and GTP, 10 μM [α-^{32}P]UTP (10 cpm/fmol), 0.2 μg of pSV01ΔEP DNA (6), 0.6 μg of T antigen, 1200 units of topoisomerase I, pol α (0.6 unit)–primase (3 units) complex, and HSSB as indicated. Samples were incubated at 37°C, phenol/chloroform-treated, and precipitated with ethanol. The precipitates were dissolved in 50 mM Tris·HCl, pH 8.5/1 mM $MgCl_2$, 0.1 mM $ZnCl_2$/1 mM spermidine. Calf intestinal alkaline phosphatase (2 units) was added and the mixture was incubated for 30 min at 37°C. Samples were then adjusted to contain 0.1 M sodium acetate (pH 5.0), 10 mM $MgCl_2$, 2 mM $CaCl_2$, 10 units of RNase inhibitor, and 1 unit of DNase I. After incubation for 60 min at 37°C, samples were phenol/chloroform-treated, ethanol-precipitated, washed in 70% ethanol (−80°C), and dissolved in 10 mM Tris·HCl, pH 7.5/1 mM EDTA containing 90% (vol/vol) formamide and tracking dye. The mixture was heated for 1 min at 90°C and loaded onto a 20% polyacrylamide gel containing 7 M urea, 1 mM EDTA, and 89 mM Tris borate (pH 8.0). Electrophoresis was done at 700 V for 5 hr with size markers. Gels were subjected to autoradiography, and regions that corresponded to radioactive bands were excised for scintillation counting.

RESULTS

Requirements for Leading-Strand Synthesis. Extensive replication of SV40 ori$^+$ DNA can be carried out with T antigen, HSSB, pol α–primase complex, and topoisomerase I (the monopolymerase system). The DNA products formed in this system, as shown by alkaline gel electrophoresis (Fig. 1, lane 3), fell into one of two well-defined size classes. In the presence of pol α–primase complex (0.4 and 1.2 units, respectively), one product resembled Okazaki fragments and was derived from the lagging-strand template, whereas the other averaged 4–5 kb (half the size of the 10-kb template) and was derived from replication of the leading-strand template. When the amount of pol α–primase complex was decreased by a factor of 20 (Fig. 1, lane 2), DNA synthesis was reduced and low levels of short chains were synthesized. Supplementation of this monopolymerase system (containing 0.02 and 0.06 unit of pol α–primase complex) with pol δ, A1, and PCNA (the dipolymerase system) resulted in the synthesis of long DNA chains and virtually no Okazaki fragments (Fig. 1, lane 6). This reaction was almost completely dependent on both T antigen and pol α–primase complex. The omission of

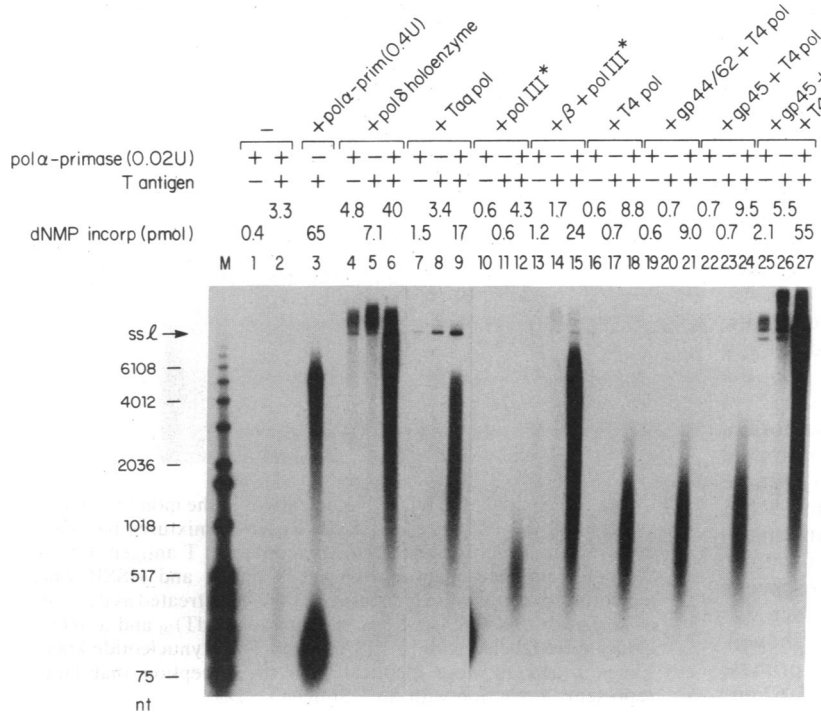

FIG. 1. Influence of various DNA polymerases on the monopolymerase system. Reaction mixtures (30 μl), as described in *Materials and Methods*, contained where indicated T antigen (0.9 μg), pol α–primase complex (0.02 and 0.12 unit, respectively), and HSSB (0.5 μg). Reactions were supplemented with the following proteins: pol α–primase complex (0.4 and 1.2 units, respectively, for the monopolymerase reaction; lane 3); 0.2 unit of pol δ, 0.15 unit of A1, and 0.12 μg of PCNA (pol δ holoenzyme; lanes 4–6); 0.4 unit of *Taq* pol (lanes 7–9); 0.16 unit of *E. coli* pol III* complex (lanes 10–12); pol III* complex plus 86 ng of β subunit (*dnaN* gene product) (lanes 13–15); 0.4 unit of T4 pol (lanes 16–18); T4 pol plus 1 μg of T4 gp44/62 (lanes 19–21); T4 pol plus 0.4 μg of T4 gp45 (lanes 22–24); T4 pol, 1 μg of T4 gp44/62, and 0.4 μg of T4 gp45 (lanes 25–27). After 60 min at 37°C, DNA synthesis was determined and is indicated above each lane, and products were analyzed as described in *Materials and Methods*. Lane M, size markers. The position of full-length linear pSVLD DNA (10 kb) is indicated as ssl. Each DNA polymerase was prepared or obtained as described in *Materials and Methods*, and the activity, with the exception of pol δ, was determined using activated DNA as the primer–template. U, unit; nt, nucleotides.

either A1 or PCNA from this system resulted in no synthesis (results not shown).

These results indicated that the pol α–primase complex was essential for the initiation of DNA synthesis. In the presence of high levels of pol α, leading-strand synthesis resulted from the elongation of the Okazaki fragments generated on the lagging strand. With low levels of pol α–primase, few Okazaki fragments were formed, and in the presence of pol δ holoenzyme they were extensively elongated. Thus, both pol α and pol δ can catalyze leading-strand synthesis.

We examined a variety of other polymerases for their ability to replace pol δ and high levels of pol α in leading-strand DNA synthesis (Fig. 1). The *E. coli* pol III* complex (lane 12) and T4 DNA pol (lane 18) partially replaced the pol δ holoenzyme. For efficient activity, the pol III holoenzyme (lane 15) and the T4 pol plus the auxiliary proteins T4 gp44/62 and T4 gp45 (lane 27) were required [also reported by Tsurimoto *et al.* (7)]. Other enzymes that supported synthesis included *Taq* pol (lane 9) and *E. coli* pol I Klenow fragment, as well as T7 DNA pol [as reported by Tsurimoto *et al.* (7)], whereas human DNA pol β did not (data not shown). Efficient DNA synthesis with these various polymerases required T antigen and the low levels of the pol α–primase complex. The activity observed in the absence of pol α–primase complex or T antigen represented end-addition to nicked pSVLD DNA (lanes 4, 5, 25, and 26).

In the SV40 mono- and dipolymerase systems, HSSB is essential for DNA replication; no other SSB replaced HSSB. Previous studies (14), as well as those described below, indicated that the pol α–primase complex is specifically stimulated by HSSB whereas the elongation reaction catalyzed by pol δ can be stimulated by a variety of SSBs. For this reason, we examined the influence of a variety of SSBs in the monopolymerase system, the dipolymerase system, and the monopolymerase system supplemented with the various DNA polymerases (Fig. 2). *E. coli* SSB, AdDBP, T4 gp32, and T7 SSB were all ineffective in supporting the replication of SV40 ori$^+$ DNA by pol α, the pol δ holoenzyme, and the T4 pol holoenzyme. Similar results were obtained with the other polymerases described above (data not shown). These results suggested that the pol α–primase reaction leading to the generation of primers for leading-strand synthesis contributed to the specific requirement for HSSB.

Specific Role of HSSB in the Primase Reaction. DNA primase catalyzes the synthesis of small RNA primers, varying in size (10, 20, and 30 nt long), that commence with ATP or GTP (15–17). In the presence of the pol α–primase complex, poly(dT) and poly(dC) support the ATP- and GTP-dependent synthesis of poly(dA) and poly(dG), respectively, whereas poly(dA) and poly(dI) [or poly(dG)] are inactive (18). The synthesis of oligoribonucleotides by primase requires single-stranded (ss) DNA, and though no specific sequence requirement appears essential for primase activity, some sequence bias has been reported (19, 20).

In a number of the prokaryotic replication systems, primase and DNA helicase activities are closely linked. T7 gp4 contains both DNA helicase and primase activities (21), the *dnaG* primase activity is linked to the *dnaB* helicase (22) in *E. coli*, and T4 gp41 (helicase) and T4 gp61 (primase) form a complex that stimulates each activity (23, 24). This prompted us to examine the influence of T antigen on HeLa primase activity on φX174 ss circular (ssc) DNA. Two small RNA products (averaging approximately 12 and 24 nt in length) were formed by primase alone, and high levels of T antigen (1.8 μg) stimulated their synthesis 2.7-fold. In the presence of HSSB, RNA synthesis was inhibited and this effect was weakly reversed by T antigen (≈2-fold; data not shown). Richardson and Nossal (25) observed that T4 DNA primase activity on φX174 ssc DNA was inhibited by T4 gp44/62 and

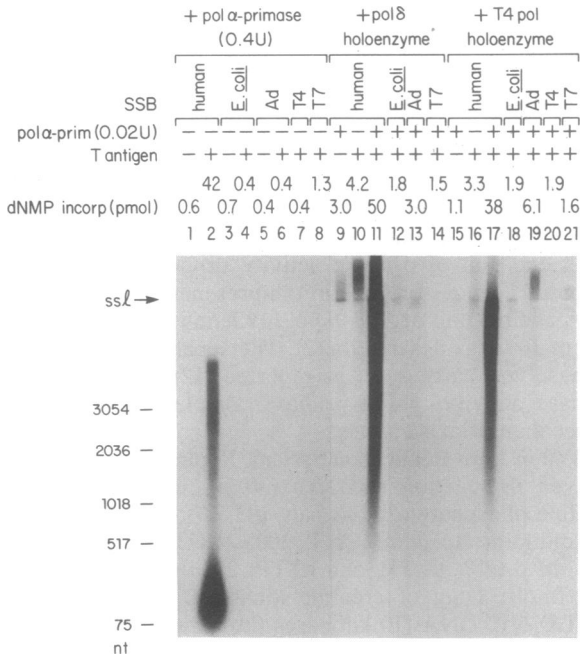

FIG. 2. Influence of various SSBs on the SV40 replication reaction with pol α, pol δ holoenzyme, and T4 pol holoenzyme. The monopolymerase system (30 μl; lanes 1–8) contained pol α–primase complex (0.4 and 1.2 units, respectively) with or without T antigen (0.9 μg). Reactions with the pol δ holoenzyme (dipolymerase system; 0.2 unit of pol δ, 0.15 unit of A1, and 0.12 μg of PCNA; lanes 9–14) or T4 holoenzyme (0.4 unit of T4 pol, 1 μg of T4 gp44/62, and 0.2 μg of T4 gp45; lanes 15–21) with or without T antigen and/or pol α–primase complex (0.02 and 0.12 unit, respectively) were as indicated. Reactions were carried out at 37°C for 60 min in the presence of 1.2 μg of HSSB (lanes 1, 2, 9–11, and 15–17), *E. coli* SSB (lanes 3, 4, 12, and 18), AdDBP (lanes 5, 6, 13, and 19), T4 gp32 (lanes 7 and 20), or T7 SSB (lanes 8, 14, and 21). DNA synthesis is indicated above each lane; DNA products were analyzed in alkaline 1.0% agarose.

that gp45 reversed this effect. For this reason, we examined the effect of T antigen on the coupled action of pol α–primase complex. The addition of HSSB, 4 pmol or 30 pmol (as

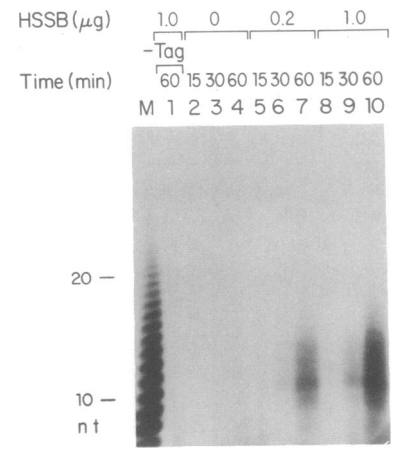

FIG. 3. Synthesis of oligoribonucleotides in the monopolymerase system and the influence of HSSB. Reaction mixtures (40 μl), as described in *Materials and Methods*, contained T antigen (0.6 μg), the pol α–primase complex (0.6 and 3 units), and HSSB where indicated. Mixtures were incubated at 37°C, then treated as described in *Materials and Methods*. For size markers, $(dT)_{10}$ and a $(dT)_{4-22}$ ladder were labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase. Lanes 1 and 10 were identical with the exception that lane 1 represents a reaction with no T antigen (−Tag).

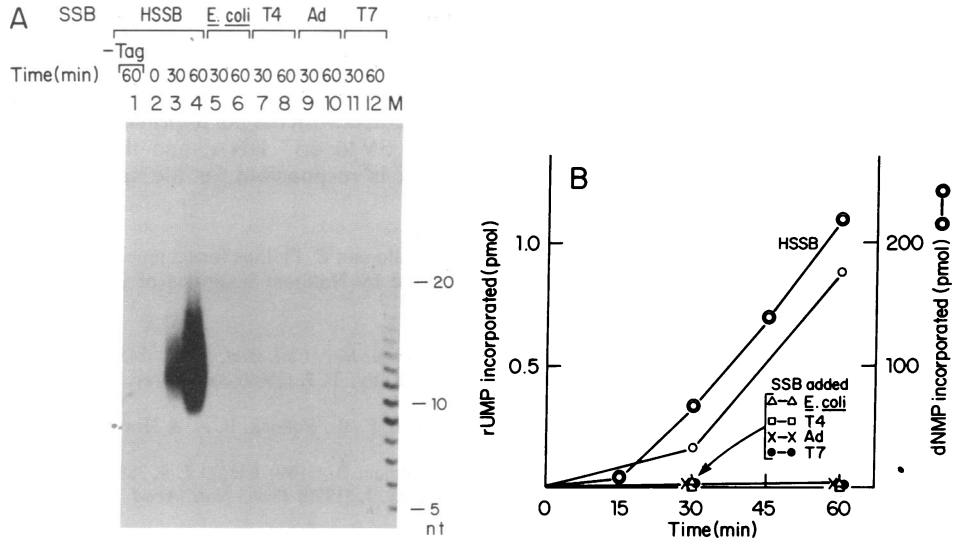

FIG. 4. Effect of various SSBs on oligoribonucleotide synthesis in the monopolymerase system. (*A*) Reactions were as described in Fig. 3 with the following additions: lanes 2–4 contained 1 μg of HSSB; lanes 5 and 6, 1 μg of *E. coli* SSB; lanes 7 and 8, 1 μg of T4 gp32; lanes 9 and 10, 1 μg of AdDBP; and lanes 11 and 12, 1 μg of T7 SSB. Mixtures were incubated for 30 or 60 min at 37°C. Lanes 1 and 4 were identical, with the exception that T antigen was omitted from lane 1. The band corresponding to oligonucleotides 8–14 nt in length was excised for scintillation counting. (*B*) A summary of these results as well as the rate of dNMP incorporation with the monopolymerase system that contained 0.4 and 1.2 units of pol α–primase complex, respectively, 0.6 μg of T antigen, and 1 μg of HSSB.

molecules to 150 pmol of φX174 ssc DNA as mononucleotide), inhibited primer-dependent DNA synthesis 70% and 100%, respectively. T antigen, in the presence or absence of A1, PCNA, and pol δ (in all combinations), did not reverse the inhibition (data not shown).

We measured primase activity in the SV40 monopolymerase reaction. In order to examine this reaction in the absence of DNA synthesis, only rNTPs were added (with [α-^{32}P]UTP) and all dNTPs were omitted (Fig. 3). After incubation and treatment with alkaline phosphatase and DNase I, products were subjected to urea/polyacrylamide gel electrophoresis. In the complete reaction, oligoribonucleotides of 8–15 nt were formed (Fig. 3, lanes 5–10) after a 15- to 30-min lag. This lag included the time required for the ATP-dependent formation of unwound DNA by T antigen, topoisomerase I, and HSSB and was similar to the lag observed in DNA synthesis (see Fig. 4*B*). In contrast to the primase reaction with φX174 ssc DNA, the primase reaction with the SV40 ori$^+$ DNA required T antigen (lane 1) and HSSB (compare lanes 2–4 with lanes 5–10) and the SV40 core origin (data not shown).

T antigen and HSSB (with topoisomerase I and ATP) are required for the unwinding of SV40 ori$^+$ DNA. Since the DNA unwinding reaction, in contrast to the monopolymerase system, works with a number of SSBs in lieu of the HSSB, experiments were performed using *E. coli* SSB, AdDBP, T4 gp32, and T7 SSB (gp2.5) in place of HSSB (Fig. 4*A*). As shown, the primase-catalyzed synthesis of oligoribonucleotide was not detected in reactions with other SSBs, compared to those with HSSB. In addition, the lag in oligoribonucleotide synthesis was similar to the lag in DNA synthesis (Fig. 4*B*). The T-antigen-catalyzed unwinding reaction was not observed with either T7 SSB or T4 gp32 (data not shown). Previous work showed that T4 gp32 did not support the unwinding reaction, whereas HSSB, *E. coli* SSB, AdDBP, and the herpes simplex virus SSB all did (14). We have assumed that the unwinding reaction catalyzed by T antigen solely required SSB for the sequestration of ss DNA. The finding that two different SSBs that efficiently bind ss DNA do not sustain unwinding suggests that this interpretation may be incomplete. It is possible that T antigen interacts with the SSB as well, and this interaction may be an important part of the unwinding reaction.

Specific Role of pol α in Supporting Primase-Catalyzed Oligoribonucleotide Synthesis. To determine whether both pol α and primase were essential for the synthesis of oligoribonucleotides in the SV40 system, experiments were repeated using separated preparations of primase and pol α. Oligoribonucleotide synthesis was drastically reduced in reaction mixtures containing primase contaminated with trace levels of pol α (0.02 unit; Fig. 5, lanes 5 and 6); oligoribonucleotide synthesis was partially restored by the addition of pol α to

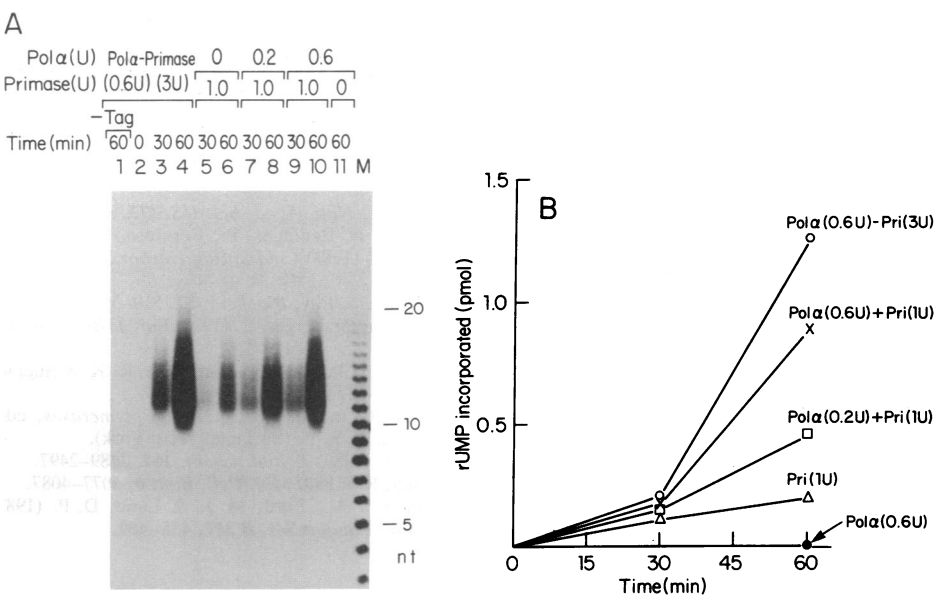

FIG. 5. Oligoribonucleotide synthesis with primase alone and primase plus pol α in the monopolymerase system. (*A*) Reactions were as in Fig. 3 and used 1 μg of HSSB. In place of purified pol α–primase complex, separated pol α and primase fractions were used. Reactions were assembled on ice, kept on ice for 60 min to permit pol α–primase complex formation, and then incubated at 37°C for the period indicated. Reactions containing pol α–primase complex are shown in lanes 2–4. In lanes 5–10, the amount of primase added was constant (1 unit containing 0.02 unit of pol α), while in lanes 5–10 the added pol α was varied (0 unit, lanes 5 and 6; 0.2 unit, lanes 7 and 8; 0.6 unit, lanes 9 and 10). Lane 11 contained 0.6 unit of pol α but no primase. (*B*) Pertinent regions of the gel represented by the autoradiogram in *A* were excised for liquid scintillation counting.

reaction mixtures containing primase (lanes 7–10). Other DNA polymerases, including *E. coli* pol I, *Taq* pol, T7 and T4 pol holoenzyme, *E. coli* pol III holoenzyme, and human pol δ holoenzyme did not replace pol α in supporting oligoribonucleotide synthesis (data not shown). No oligoribonucleotide synthesis was observed in the absence of T antigen (lane 1) or primase (lane 11). The monopolymerase system is inhibited by poly(ADP-ribose) polymerase, A1, and A1 plus PCNA. However, these proteins did not affect oligoribonucleotide synthesis in this system (data not shown). These observations suggest that primase alone initiates primer synthesis poorly, if at all, in the absence of pol α.

DISCUSSION

We have demonstrated that the replication of SV40 ori$^+$ DNA can be carried out by a number of different DNA polymerases, provided that the reaction mixture contains the monopolymerase system (T antigen, HSSB, and the pol α–primase complex). The extent of replication with this system is influenced by the amount of pol α–primase complex. In the presence of high levels of the complex, both short Okazaki fragments, arising from lagging-strand replication, and long DNA products, arising from replication of the leading strand, were produced (6). With low levels of pol α–primase complex, DNA replication required the presence of pol δ and its auxiliary proteins A1 and PCNA. The pol δ holoenzyme can efficiently elongate low concentrations of DNA primers generated by low levels of the pol α–primase complex. Other DNA polymerases can replace the pol δ holoenzyme and can efficiently elongate the minute amounts of primer ends formed. As in the eukaryotic system, the most effective polymerases examined were the *E. coli* pol III holoenzyme and the T4 pol holoenzyme, two multiprotein elongation complexes that interact with low levels of primer ends. These prokaryotic systems are also highly processive in their action (26–30). A more detailed study of the processivity of the pol δ holoenzyme system has not yet been carried out.

In prokaryotic systems one DNA polymerase supports both leading- and lagging-strand synthesis, while in the eukaryotic systems there are more than one. It is likely that pol α, by virtue of its strong interaction with primase (17), is involved in the initiation of DNA chains for both leading and lagging strands, and that the pol δ holoenzyme is involved in the elongation of the chains initiated by the pol α–primase complex. Recently, a third DNA polymerase, pol ε [pol II in yeast (31)] has been shown to be essential for DNA synthesis in yeast (32, 33). The counterpart of yeast pol ε has been isolated from HeLa cells by Syvaoja and Linn (34). The HeLa enzyme as well as the yeast pol ε were considered to be PCNA-independent enzymes. However, in the presence of salt, both pol ε preparations were found to be completely dependent on A1 and PCNA (P. M. J. Burgers, S.-H. Lee, Z. Q. Pan, and J.H., unpublished observation). Thus, two different polymerases may be involved in the elongation of Okazaki fragments. How pol α and the other DNA polymerases interact to control leading- and lagging-strand synthesis at the replication fork is unknown. Further work on the proteins at the replication fork should be more illuminating.

The action of the pol α–primase complex in the initiation of primer synthesis must be influenced by the interaction of the complex with T antigen. The interaction of pol α and T antigen has been reported by Smale and Tjian (35) and by Mole *et al.* (36). The large double hexamer of T antigen at the SV40 origin, if available for binding, could position pol α–primase. The ATP-dependent unwinding reaction catalyzed by T antigen occurs after a pronounced lag, as does oligoribonucleotide synthesis. An important feature of the priming reaction is its specificity for pol α and HSSB. Since unwinding can be carried out by T antigen in the presence of a variety of SSBs, the unwinding reaction *per se* is not the essential reaction for the initiation of primer synthesis. It is possible that HSSB interacts with the pol α–primase complex and T antigen on the SV40 ori$^+$ DNA, and this multiple protein–DNA complex is responsible for the specificity in primer synthesis.

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