JOURNAL OF VIROLOGY, Dec. 1990, p. 5912–5918
0022-538X/90/125912-07$02.00/0

Vol. 64, No. 12

Two DNA Polymerases May Be Required for Synthesis of the Lagging DNA Strand of Simian Virus 40

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Received 23 May 1990/Accepted 20 August 1990

Agents discriminating between DNA polymerase α and DNA polymerases of class δ (polymerase δ or ε) were used to characterize steps in the synthesis of the lagging DNA strand of simian virus 40 during DNA replication in isolated nuclei. The synthesis of lagging-strand intermediates below 40 nucleotides, termed DNA primers (T. Nethanel, S. Reisfeld, G. Dinter-Gottlieb, and G. Kaufmann, J. Virol. 62:2867–2873, 1988), was selectively inhibited by butylphenyl dGTP or by neutralizing DNA polymerase α monoclonal antibodies. The synthesis of longer lagging chains of up to 250 nucleotides (Okazaki pieces) was affected to a lesser extent, possibly indirectly, by these agents. Aphidicolin, which inhibits both α- and δ-class enzymes, elicited the opposite pattern: DNA primers accumulated in its presence and were not converted into Okazaki pieces. These and previous data suggest that DNA polymerase α primase synthesizes DNA primers, whereas another DNA polymerase, presumably DNA polymerase δ or ε, mediates the conversion of DNA primers into Okazaki pieces.

Many lines of evidence now support the involvement of two DNA polymerases, α and δ (pols α and δ, respectively), in the replication of eucaryotic and simian virus 40 (SV40) chromosomes (5, 19, 25). The two enzymes are distinguished by their associated activities: pol α is in a complex with primase (19), and pol δ has an intrinsic 3′-to-5′-proofreading exonuclease (6, 25). pols α and δ also differ in chromatographic behavior (6), template primer preference (6), and antigenic properties (9, 17, 31). The nucleotide analogs butylphenyl-dGTP (BuPdGTP) and butylanilino-dATP are potent inhibitors of pol α, whereas pol δ is relatively resistant to these compounds (18, 25). On the other hand, the antibiotic aphidicolin inhibits both enzymes (25).

Two different mammalian DNA polymerases associated with a 3′-exonuclease and resistant to BuPdGTP were previously referred to as pol δ. These enzymes, later distinguished as pols δ1 and δ2, are termed now pols δ and ε, respectively (P. M. Burgers et al., Eur. J. Biochem., in press). An important difference between the two enzymes is that pol δ depends on the proliferating nuclear cell antigen (PCNA) for its full activity and processivity, whereas pol ε is processive in the absence of PCNA and is not stimulated by the addition of this ancillary protein (5). Since PCNA is needed for SV40 DNA replication in vitro, pol δ is implicated in the process (22). Direct evidence on this point has been provided by experiments in which both pol α primase and the PCNA-dependent pol δ were required to reconstitute an in vitro SV40 DNA replication system (30). pol ε has been implicated in DNA repair (26), but whether it also has a replicative role is not known.

The distinction between the three aphidicolin-sensitive mammalian DNA polymerases, pols α, δ, and ε, is supported by a comparison with the corresponding set of *Saccharomyces cerevisiae* enzymes (reviewed in reference 5). pol α and its *S. cerevisiae* homolog pol I share the same subunit structure. An essential cell cycle gene, *CDC17*, encodes the large polymerase subunit of pol I and features an extensive similarity with the corresponding mammalian gene at the amino acid level (15, 21, 32). *S. cerevisiae* pol III, whose major polymerase subunit is encoded by the *CDC2* gene (4, 24), is analogous with mammalian pol δ. This conclusion is based on the similar subunit structure of the two enzymes and the ability of pol III to interact equally well with mammalian and *S. cerevisiae* PCNAs (2, 5). The deduced amino acid sequence of the large polymerase subunit of pol III shows a greater resemblance to those of Epstein-Barr virus and herpes simplex virus DNA polymerases than to the pol α and pol I sequences, thus defining two prototypes of related families of eucaryotic DNA polymerases (4, 5). Less is known about pol II, the *S. cerevisiae* analog of the mammalian pol ε. A gene encoding pol II has not been identified. However, a *ts-cdc2* mutant is devoid of pol III but expresses normal levels of pol II at the restrictive temperature, indicating that the two *S. cerevisiae* enzymes and, by implication, the mammalian pols δ and ε, are genetically distinct (4, 5, 25).

The diversity of replicative eucaryotic DNA polymerases is explained by a recent model that assigns synthesis of the lagging DNA strand to pol α and synthesis of the leading strand to pol δ (3, 5, 11, 23). This division of labor is consistent with the high processivity of pol δ in the presence of PCNA, a property expected of a leading-strand polymerase (22). In agreement, removal of PCNA from an SV40 DNA replication system arrests leading-strand DNA synthesis, whereas lagging-strand synthesis proceeds uncoupled (23). On the other hand, the moderately processive pol α, with its tightly associated primase, seems suited for periodic priming and elongation of the short lagging DNA chain intermediates.

Although this model satisfies the expectation of different requirements for leading- and lagging-strand syntheses, the actual replication mechanism could be more complex with regard to the number of specific replicative reactions and different DNA polymerases catalyzing them. We have previously proposed that the synthesis of the lagging DNA strand of SV40 consists of two steps that differ in sensitivity to aphidicolin (20). In the less sensitive step, an RNA primer (initiator RNA [iRNA]) chain is extended by several tens of deoxynucleoside monophosphates (dNMPs) into an intermediate termed the DNA primer. In the more sensitive step, DNA primers are converted into longer chains of up to

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approximately 250 nucleotides (nt), termed Okazaki pieces. We have further proposed, based on the configuration of DNA primers in replicating DNA, that an Okazaki piece arises by ligation of several adjacent DNA primer units (20; see also an updated version of the nested discontinuity model in Fig. 7). Accordingly, a DNA polymerase that is relatively resistant to aphidicolin extends iRNA into a DNA primer. Another polymerase that is more sensitive to aphidicolin fills gaps created between DNA primer units by the prior removal of intervening iRNA moieties.

In the present communication we extend the investigation on the two steps of lagging-strand DNA synthesis to include two specific inhibitors of pol α. We show that BuPdGTP and neutralizing pol-α monoclonal antibodies selectively inhibit the synthesis of DNA primers and have a lesser effect on the aphidicolin-sensitive synthesis of Okazaki pieces. These results are consistent with the idea that pol α primase catalyzes only DNA primer synthesis, whereas another polymerase, presumably pol δ or ε, mediates the conversion of DNA primers into Okazaki pieces.

MATERIALS AND METHODS

Materials. BuPdGTP was a gift from George Wright, University of Massachusetts Medical School, Worcester. Aphidicolin was purchased from Sigma. DNA pol α-specific monoclonal antibodies SJK 132-20, 287-38, and 237-71 (28) and mouse myeloma immunoglobulin G were purchased from Pharmacia. Enzymes were purchased from New England BioLabs, and α-amanitin was from Boehringer. Radioactive compounds and GeneScreen Plus membranes were purchased from NEN Research Products.

SV40 DNA synthesis in isolated nuclei. In vitro SV40 DNA synthesis was performed in isolated nuclei supplemented with cytosol; both fractions were isolated from infected CV-1 African monkey kidney cells (20). Unless otherwise stated, unlabeled deoxynucleoside triphosphates (dNTPs) were employed at 2 μM; the labeled dNTPs were used at 0.5 to 1.0 μM, ATP was used at 1 mM, and rNTPs other than ATP were used at 10 μM or at 1 μM when radioactively labeled. In the latter case, α-amanitin was used to quench transcriptional incorporation (16). The isolated SV40 replicative-intermediate DNA was of the post-Sepharose gel filtration fraction (20).

Fractionation and analysis of nascent DNA chains. Isolated SV40 replicative-intermediate DNA was dissociated in formamide, and the nascent DNA was fractionated by denaturing polyacrylamide electrophoresis along with DNA size markers. The gels were autoradiographed, and the distribution of radioactivity as a function of DNA chain length was quantified by densitometry (20). Nascent DNA chain fractions of the indicated size classes were extracted from the gel and analyzed for replication-fork polarity by hybridization to the appropriate unidirectional single-stranded DNA M13-SV40 probes immobilized on dot blots (20). The dot blots were prepared by applying the DNA in 0.4 M NaOH to GeneScreen membrane as described by Jagus (14) and hybridized according to the instructions of the manufacturer in hybridization solution containing 500 to 2,000 cpm of ^{32}P-labeled DNA. After hybridization the blots were washed twice with 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 25°C for 10 min and once with 2× SSC and 1% sodium dodecyl sulfate for 60 min at 65°C and then autoradiographed. The iRNA moiety in nascent DNA chains labeled from radioactive RNA precursors was sized by DNase I digestion (16).

RESULTS

Opposite effects of BuPdGTP and aphidicolin on steps of lagging DNA synthesis. In the system of isolated nuclei used in this study, SV40 replication cycles initiated in vivo continue in vitro. Initiation of new replication cycles is not detected in this system (29). Hence, the system allows attention to be focused on elongation of leading and lagging DNA chains and priming of new Okazaki pieces. Overall SV40 DNA synthesis in isolated nuclei was relatively resistant to BuPdGTP; only 50% inhibition was observed at 50 μM of the drug during a pulse of 2 min at 30°C. The system of isolated nuclei resembles other SV40 and cellular replication systems in this regard (7, 10, 13) but differs from purified pol α enzymes, which are inhibited by BuPdGTP with K_i values in the nanomolar range (18), and from pol δ, which is resistant to BuPdGTP at concentrations as high as 100 μM (18).

The poor response of the replicative systems to the pol α-specific inhibitor BuPdGTP could mean that the contribution of pol δ to replicative DNA synthesis is greater than that of pol α (1, 7). Alternatively, within the replication complex individual DNA polymerases may behave differently from the corresponding uncoupled purified enzymes. In an attempt to determine the specific contributions of individual DNA polymerases to SV40 DNA replication, we compared the effect of BuPdGTP on leading- versus lagging-strand synthesis and, within the latter category, on DNA primer versus Okazaki piece synthesis.

The size distribution of nascent SV40 DNA chains pulse-labeled with [α-^{32}P]dNTPs in the presence of BuPdGTP at concentrations ranging between 10 and 100 μM differed markedly from that of the control. This was indicated by electrophoretic separation of the products on denaturing polyacrylamide gels (Fig. 1). Specifically, the incorporation into the long nascent chains (above 250 nt) was virtually resistant to BuPdGTP during the 2-min pulse at 30°C. Within the subpopulation of chains below 250 nt, BuPdGTP was more inhibitory to the synthesis of the DNA primer fraction (up to 40 nt) than to the synthesis of Okazaki pieces (40 to 250 nt) (Fig. 1, lanes 3 through 5). The residual BuPdGTP-resistant incorporations were nearly abolished by further addition of 10 μg of aphidicolin per ml (Fig. 1, lane 7). When added alone at 10 μg/ml, aphidicolin inhibited 70 to 90% of the overall incorporation in different experiments. The aphidicolin-resistant synthesis consisted of mainly DNA primers and long nascent chains above 250 nt (Fig. 1, lane 6) (20), in marked contrast to the effect of BuPdGTP (compare lanes 5 and 6 in Fig. 1). However, the incorporation of DNA precursors into the DNA primer fraction was only relatively resistant to aphidicolin, compared with the synthesis of Okazaki pieces. Increasing the concentration of aphidicolin up to 100 μg/ml gradually reduced the synthesis of DNA primers (Fig. 2). It is also noteworthy that the level of the longest Okazaki pieces remained unchanged between 5 and 100 μg of aphidicolin per ml; an aphidicolin-resistant subfraction of the long nascent DNA behaved in a similar manner.

BuPdGTP blocks de novo synthesis of DNA primers. We examined more closely the effects of BuPdGTP and aphidicolin on newly synthesized DNA primers that were tagged in their 5′ iRNA moiety with [α-^{32}P]rUTP. During 2 min of incubation in the absence of inhibitors, most of the label appeared as full-sized DNA primers (Fig. 3, lane 1). Longer incubations resulted in increased labeling of Okazaki pieces (Fig. 3, lanes 2 and 3). In the presence of 10 μM BuPdGTP,

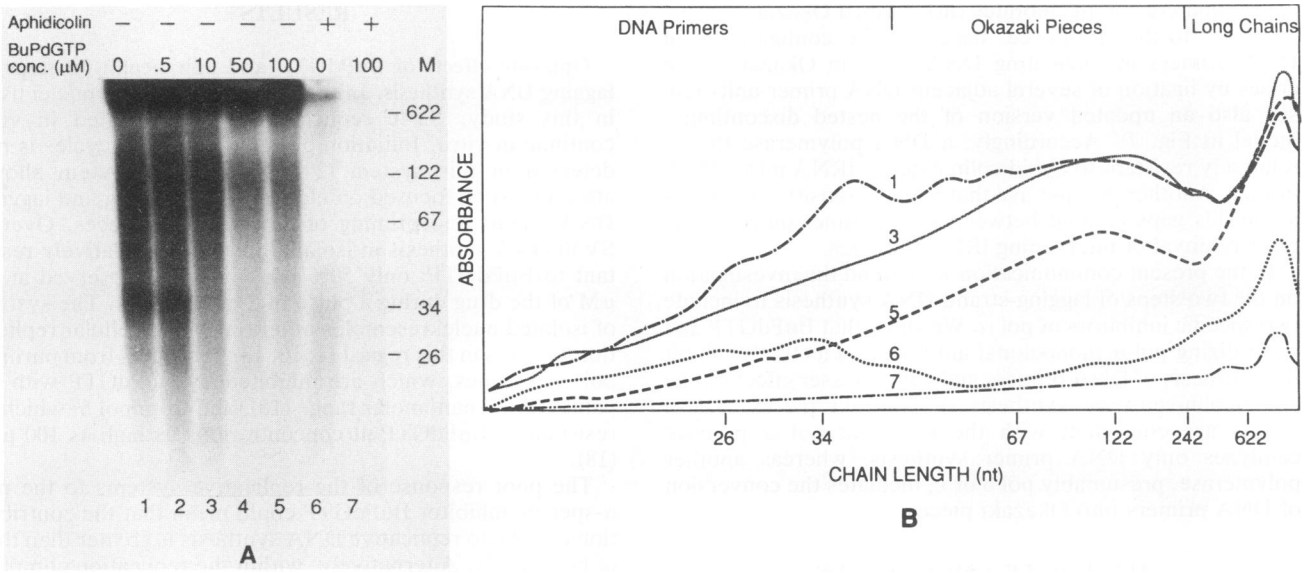

FIG. 1. BuPdGTP selectively inhibits the incorporation of dNMPs into DNA primers. SV40 replicative-intermediate DNA synthesized in isolated nuclei was pulse-labeled for 2 min at 30°C with[α-^{32}P]dATP in the presence of the indicated concentrations of BuPdGTP and/or 10 μg of aphidicolin per ml. The labeled SV40 replicative-intermediate DNA was isolated as described in Materials and Methods and dissociated in formamide. The nascent DNA chains were fractionated by denaturing 12% polyacrylamide gel electrophoresis. (A) Autoradiogram. (B) Densitometric tracing of lanes 1, 5, 6, and 7 in panel A. M, DNA size markers in nucleotides (*Msp*I restriction fragments of pBR322 DNA). Okazaki piece/DNA primer ratios computed from the densitometric tracing: without inhibitor (lane 1), 1.6; with 10 μM BuPdGTP (lane 3), 2.1; with 100 μM BuPdGTP (lane 5), 2.9; with 10 μg of aphidicolin per ml (lane 6), 0.7.

most of the iRNA-tagged DNA chains could not reach the size of mature DNA primers, migrating instead as two major peaks of approximately 18 and 20 nt (Fig. 3, lanes 4 through 6). That the iRNA-tagged DNAs were lagging-strand intermediates was indicated by their fork polarity (Fig. 4B) and by the fact that they could be digested with DNase I down to an iRNA-like residue of approximately 10 nt (Fig. 5). The BuPdGTP-resistant intermediates yielded in this analysis an iRNA moiety that was identical with the product obtained from DNA primers synthesized in the absence of inhibitor (Fig. 5, lane 4), indicating that the inhibitor interfered with the extension of iRNA. At 50 μM BuPdGTP, no iRNA-tagged DNA chains were detected (data not shown). In contrast to BuPdGTP, aphidicolin only slowed down the formation of DNA primers. Prolonged incubation in the presence of aphidicolin resulted in the accumulation of full-sized DNA primers. Okazaki pieces were not synthesized under these conditions (Fig. 3, lanes 7 through 9) (20).

BuPdGTP and aphidicolin do not block the joining of Okazaki pieces onto long nascent chains. Since Okazaki pieces could be still synthesized in the presence of high concentrations of BuPdGTP, we expected that they would also mature and join onto the 5′ ends of long nascent chains under these conditions. In this case, the nascent portions of long chains labeled in the presence of BuPdGTP would have comprised both leading and lagging sequences. In contrast, the residual incorporation into the long chains that occurred in the presence of aphidicolin was expected to be confined to leading portions, since synthesis of Okazaki pieces was inhibited under these conditions. These possibilities were examined by comparing the replication fork polarities of the control long chains to their BuPdGTP- or aphidicolin-resistant counterparts by hybridization to suitable leading and lagging SV40 DNA template probes (see Materials and Methods). These nascent DNA chains were fragmented before hybridization to separate their leading and lagging parts (20). The BuPdGTP-resistant long nascent DNA fraction consisted of both leading and lagging portions, similar to the control long chains (Fig. 4A). These results suggested that the elongation of the leading 3′ ends and the maturation and joining of Okazaki pieces to the 5′ ends of the long chains continued in the presence of BuPdGTP, at least during the 2-min pulse. Surprisingly, the residual, aphidicolin-resistant incorporation into long chains also comprised a significant portion of lagging sequences (Fig. 4A). The possible meaning of this result is discussed below.

Two different pol α-specific monoclonal antibodies selectively inhibit DNA primer synthesis. The pol α-specific neutralizing monoclonal antibodies SJK 132-20 and SJK 287-38 inhibited DNA primer synthesis selectively. Okazaki piece synthesis was less affected, whereas long nascent chains were not affected at all (Fig. 6, lanes 1 and 2). This pattern was strikingly similar to that elicited by BuPdGTP (Fig. 1). Antibody SJK 132-20 was more potent than SJK 287-38, in keeping with the different degrees of inhibition that these antibodies exert over isolated pol α (28). Importantly, SJK 132-20 has no detectable cross-reactivity with human pol δ or with other pol δ enzymes from phylogenetically diverse vertebrates (31). The pol α binding (but not neutralizing) antibody SJK 237-71 and mouse immunoglobulin G had no effect on DNA synthesis (Fig. 7, lanes 3 and 4).

DISCUSSION

The foregoing data reinforce the idea of two distinct steps in the synthesis of the lagging DNA strand: (i) extension of iRNA by a few tens of dNMPs to generate a DNA primer and (ii) conversion of DNA primers into an Okazaki piece (20). These data also suggest the assignment of a different DNA polymerase to each step (Fig. 7). Below we elaborate these conclusions and discuss the evidence upon which they are based.

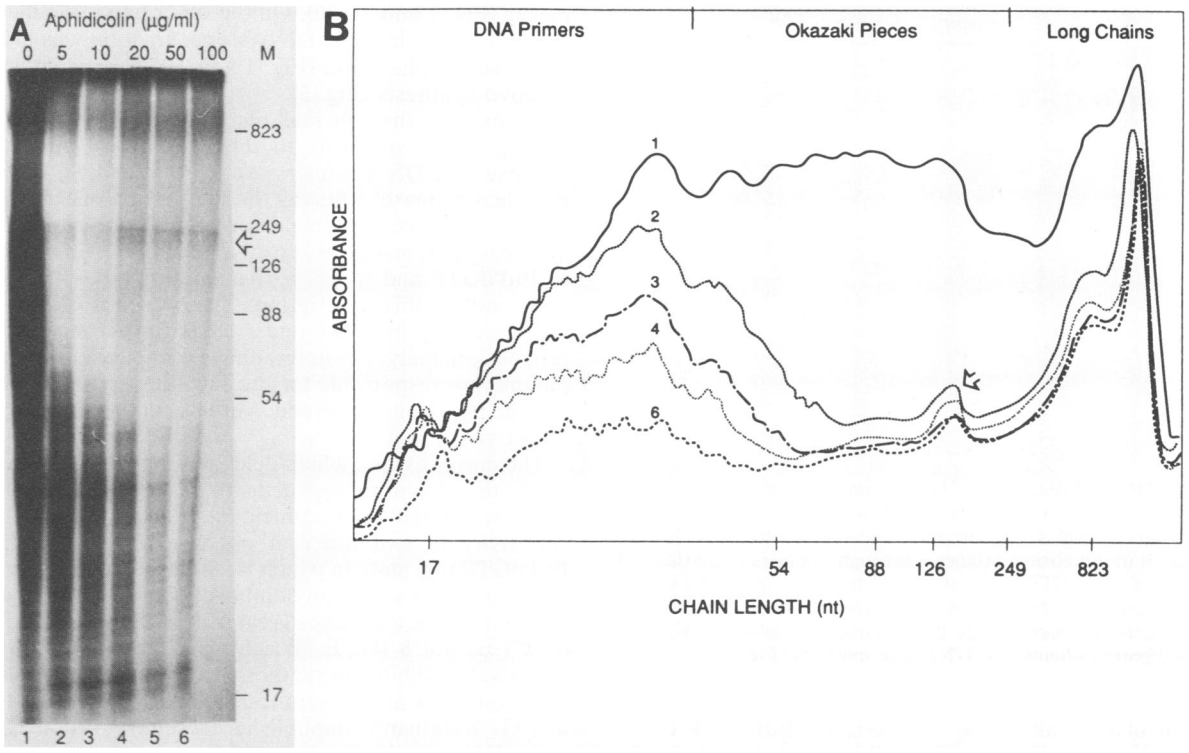

FIG. 2. Size distribution of nascent SV40 DNA as a function of aphidicolin concentration. SV40 replicative-intermediate DNA synthesized in isolated nuclei was labeled in the presence of the indicated concentrations of aphidicolin and fractionated in a manner similar to that described in the legend to Fig. 1, except that the top third of the gel contained 8% polyacrylamide to resolve chains above 250 nt. (A) Autoradiogram. The arrow points at the fraction of longest Okazaki pieces. (B) Densitometric trancing of lanes 1 through 4 and 6 in panel A. M, DNA size markers (*Bst*NI restriction fragments of SV40 DNA).

Agents that specifically inhibit isolated DNA polymerase α also exerted a specific effect on the synthesis of DNA primers during SV40 DNA replication in isolated nuclei. Thus, BuPdGTP selectively inhibited the synthesis of DNA primers, having a lesser effect on the incorporation of dNMPs into Okazaki pieces (Fig. 1, 3, and 4). The partial

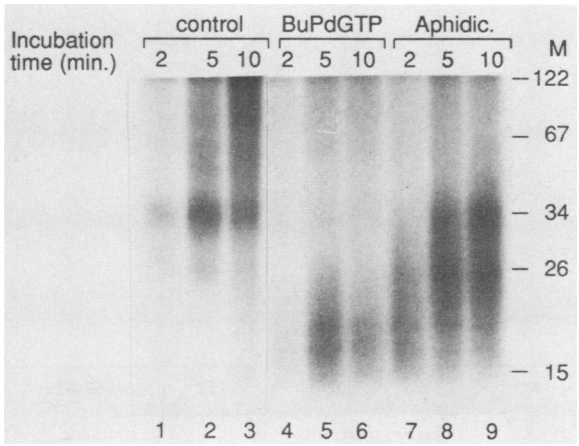

FIG. 3. BuPdGTP blocks de novo synthesis of DNA primers. SV40 replicative-intermediate DNA was labeled for the indicated times with [α-^{32}P]UTP in the absence or presence of the indicated inhibitors. The nascent DNA was fractionated as described in the legend to Fig. 1.

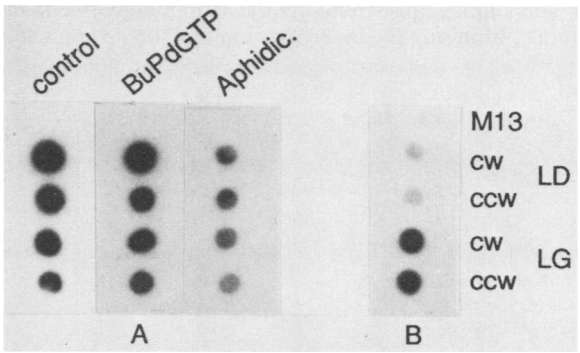

FIG. 4. Replication fork polarities of nascent DNA fractions. (A) Fork polarity of long nascent DNA synthesized in the presence of BuPdGTP or aphidicolin. Long DNA chains (Fig. 1) synthesized in the absence of inhibitors (lane 1) or in the presence of BuPdGTP (lane 4) or aphidicolin (lane 6) were fragmented in the gel, eluted, and hybridized to 1.5-μg dots of single-stranded DNA of M13-SV40 clones serving as unidirectional probes (20) (see Materials and Methods). M13, M13mp10 single-stranded DNA; ccw and cw, counterclockwise and clockwise replication fork probes, respectively; LD and LG, leading and lagging templates, respectively. The ratio of leading templates to lagging templates was computed by two-dimensional densitometric tracing of the corresponding dots. The respective values for clockwise and counterclockwise forks, respectively, were as follows: control, 1.8 and 2.0; BuPdGTP, 2.0 and 1.7; aphidicolin, 1.0 and 2.4. (B) Fork polarity of iRNA-tagged DNA primers. DNA primers (Fig. 3, pooled lanes 1 through 3) were extracted from the gel and hybridized to the probes described above. The leading/lagging template ratio was 0.1 for both forks.

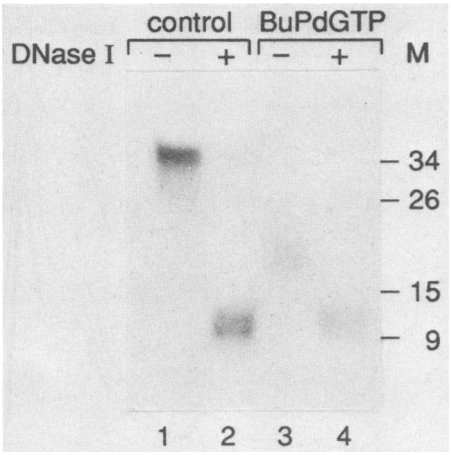

FIG. 5. DNase I digestion of DNA primer intermediates synthesized in the absence or presence of BuPdGTP. The major bands of DNA primers or DNA primer intermediates labeled with [α-^{32}P] UTP (Fig. 3) in the absence (lanes 1 through 3) or presence (lanes 4 through 6) of BuPdGTP were extracted from the gel and exhaustively digested with DNase I (16). The products were separated by 20% denaturing polyacrylamide gel electrophoresis along with the undigested parent chains. M, DNA size markers (Fig. 1).

inhibition of Okazaki piece synthesis by BuPdGTP could reflect an indirect effect of this inhibitor; namely, that only DNA primers completed before the addition of BuPdGTP may have served as substrates in the conversion reaction. Similar to BuPdGTP were two different pol α-neutralizing antibodies that preferentially inhibited the incorporation of dNMPs into DNA primers (Fig. 6).

Aphidicolin, the diagnostic inhibitor of the replicative pol α and δ as well as of pol ε (12, 18), discriminated between the two steps in lagging-strand synthesis in a converse manner, severely inhibiting the incorporation of dNMPs into Okazaki pieces (Fig. 1 and 2) (20). On the other hand, the incorporation of dNMPs into the DNA primer fraction was much less sensitive to aphidicolin (Fig. 1 and 2) (20), and so was their de novo synthesis (Fig. 3).

To explain the unequal responses of the two steps in lagging-strand synthesis to this variety of inhibitors, we propose that DNA primers are synthesized by DNA polymerase α-primase, whereas the conversion of DNA primers into Okazaki pieces depends on another DNA polymerase. The latter polymerase is sensitive to aphidicolin but resistant to BuPdGTP and to pol α-specific antibodies. Both pols δ and ε satisfy this description. The presence of a 3′-exonuclease activity in pols δ and ε lends further support to this proposal; namely, a proofreading exonuclease present in the polymerase responsible for the second step could safeguard the fidelity of lagging-strand synthesis in the absence of such an activity in pol α.

The current view, which delegates lagging DNA synthesis solely to pol α-primase, seems to us less likely, since it has to be reconciled with coincident transition of pol α from sensitivity to two different pol α-specific antibodies and BuPdGTP to a state in which it is refractory to these agents. We also note that carbonyldiphosphonate, which inhibits pol ε with a potency 20 times greater than that displayed for pol α (27) or pol δ (G. E. Wright, personal communication), selectively inhibits the synthesis of Okazaki pieces with little effect on DNA primer synthesis (T. Nethanel, C. McKenna, and G. Kaufmann, unpublished results). Although aphidicolin does not clearly distinguish between pols α, δ, and ε when compared by the same assay, these enzymes may feature different responses to the inhibitor when engaged in different replicative reactions.

Based on the results presented here, we cannot state which of the two enzymes, pol δ or ε, is more likely to figure in the second step of lagging-strand synthesis. The PCNA-dependent pol δ was proposed as the leading-strand polymerase, since depletion of PCNA from the in vitro SV40 DNA replication system confined DNA synthesis to lagging-

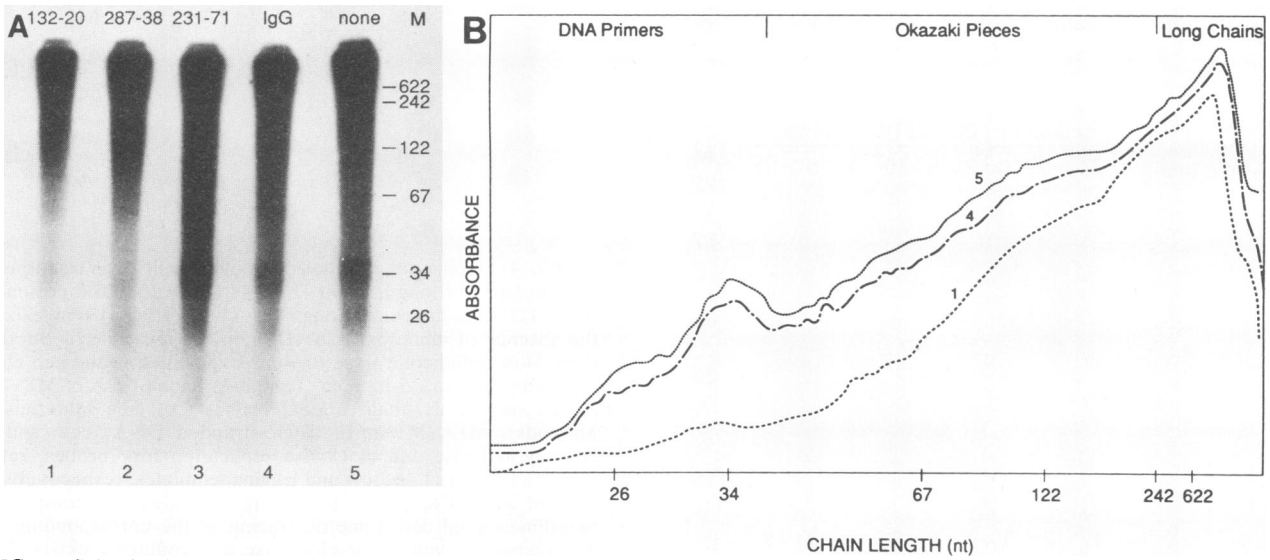

FIG. 6. Selective inhibition of DNA primer synthesis by pol α-specific antibodies. Nuclei and cytosol were separately incubated for 1 h at 0°C with the indicated antibody or mouse immunoglobulin G (IgG) (10 μg per 80 μl of cytosol or 40 μl of suspended nuclei) and then combined with the other components of the in vitro replication mixture. SV40 replicative-intermediate DNA was then labeled with [α-^{32}P]dGTP for 5 min at 30°C, isolated, and fractionated as described in the legend to Fig. 1. (A) Autoradiogram. (B) Densitometric tracing of lanes 1, 4, and 5 of panel A. M, DNA size markers (Fig. 1).

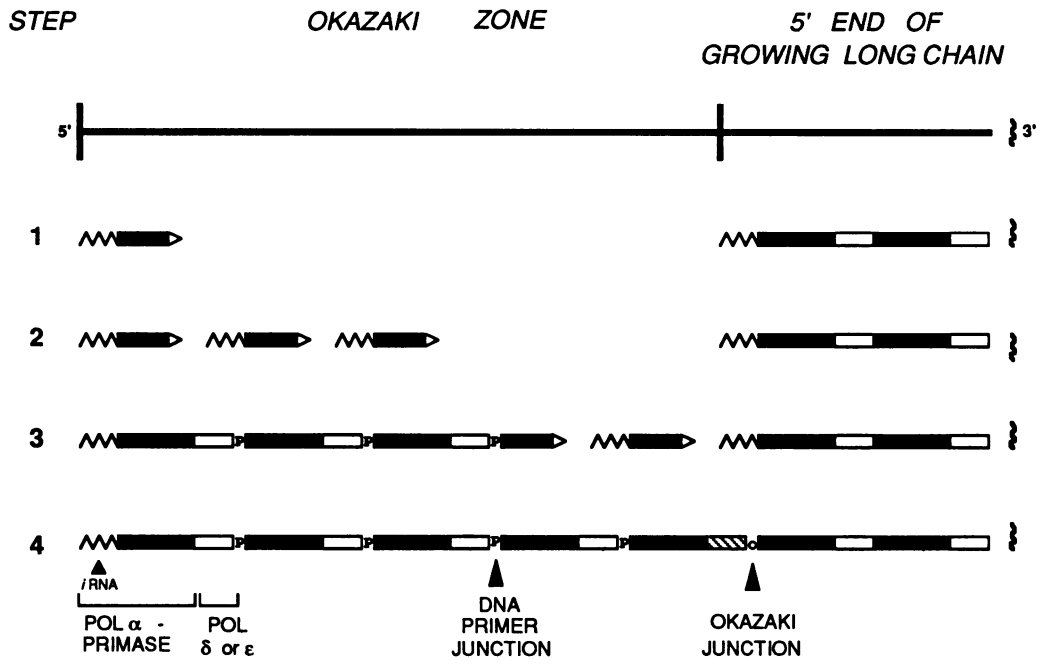

FIG. 7. Model for synthesis of the lagging strand of SV40. Symbols: ∿, iRNA; ∿▬, iRNA extended with DNA primer portion synthesized by pol α; ∿▬▭, DNA primer with downstream gap created by iRNA removal filled by pol δ or ε; ▨, former gap between a mature Okazaki piece and the growing 5′ end of the long chain; p, DNA primer ligation junction; o, Okazaki piece and long-chain ligation junction.

strand products the size of Okazaki pieces (23). Yet the leading-strand assignment of pol δ does not preclude a role in lagging-strand synthesis. That is, the reduced processivity of pol δ in the absence of PCNA may still suffice for converting DNA primers into Okazaki pieces. Alternatively, coordinated syntheses of leading and lagging DNA are mediated by pols δ and α, whereas pol ε follows behind, filling gaps between adjacent DNA primer units in a repairlike activity characteristic of this enzyme (26). The latter possibility is favored by the effect of carbonyldiphosphonate, which is cited above.

Nascent lagging-strand sequences were found in long DNA chains labeled in the presence of aphidicolin (Fig. 4A), in apparent conflict with the assumption that under these conditions Okazaki pieces cannot mature and, hence, do not join onto long chains. Yet the effect of aphidicolin on the incorporation of dNMPs into Okazaki pieces was not uniform across the 40 to 250-nt range. A residual amount of label was incorporated into a narrow subpopulation of the longest Okazaki pieces at aphidicolin concentrations even as high as 100 μg/ml (Fig. 2). A possible explanation of this result is that the gap-filling reaction between adjacent DNA primer units is more sensitive to aphidicolin than that which precedes the joining of a mature Okazaki piece to the 5′ end of a growing long chain (Fig. 7). Previous reports have shown that the latter gap-filling reaction is a distinct event that depends on factors not essential to the synthesis of the Okazaki piece itself (8).

The incorporation of dNMPs into the long nascent DNA fraction above 250 nt was only marginally affected by BuPdGTP at concentrations as high as 100 μM during a pulse of 2 min (Fig. 1). The nascent parts of these chains contained both leading and lagging DNA sequences (Fig. 4A). Hence, the elongation of leading 3′ ends of long chains and the joining of Okazaki pieces to their 5′ ends must have continued in the presence of BuPdGTP. Taken together with the low sensitivity of these synthetic steps to the two pol α-neutralizing antibodies (Fig. 6), these data are consistent with the postulated role of pol δ in leading-strand synthesis (2, 19) and the role proposed here for pol δ or ε in lagging-strand synthesis.

In summary, both pol α primase and a class-δ DNA polymerase may be engaged in synthesis of the lagging strand of SV40 and, by implication, of eucaryotic chromosomes. We wish to emphasize that these assignments are tentative, being based on correlations of inhibitor effects on purified enzymes on the one hand with discrete replicative reactions on the other. Further studies employing reconstituted systems and DNA polymerase mutants will be required to test the proposed enzymatic assignments and their physiological validity and general applications. In Fig. 7 we incorporate suggestions made above in an updated version of the previously proposed model of a nested discontinuity in lagging-strand synthesis (20). In the current version we assign to pol α primase the function of DNA primer synthesis and to pol δ or ε the role of gap filling between adjacent DNA primer units. The gap-filling reaction that precedes the joining of an Okazaki piece to the 5′ end of a long nascent chain is marked as a distinct event. The 5′-to-3′ direction of overall growth of an Okazaki piece is suggested by the occurrence of gaps up to 200 nt long downstream to DNA primers arrested in the presence of aphidicolin (T. Nethanel, T. Zlotkin, and G. Kaufmann, unpublished data).

ACKNOWLEDGMENTS

We thank George E. Wright for making results available before publication, and we gratefully acknowledge the gift of BuPdGTP. We thank Shlomo Eisenberg, Michael Fry, Joel Huberman, Rolf Knippers, and Joseph Shlomai for critical reading of the manuscript. We thank Michal Amitsur, Daphne Chapman, David Potter, and

Tamar Zlotkin for useful comments and Angella Cohen for help in the preparation of this manuscript.

This work was supported by grants from the United States-Israel Binational Science Foundation, Jerusalem, Israel, and from the Israel Cancer Research Fund.

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