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Mouse primase initiation sites in the origin region of simian virus 40

(initiation of DNA synthesis/*in vitro* initiation/RNA priming)

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ABSTRACT **The sites of initiation of DNA synthesis by purified mouse DNA primase in the origin-of-replication region of simian virus 40 (SV40) were examined. Using as template the separated strands of a cloned fragment of SV40 ≈300 base pairs (bp) long that includes the origin, we observed specific sites of initiation on the two strands. On the early strand that is the template for early mRNA synthesis, the primary starts are at four positions within 10 nucleotides of each other around nucleotide 5215 and an additional site around nucleotide 5147 that is used at one-sixth the frequency of the major sites. The major start sites on the early strand are within the 65-bp minimal origin of replication and lie between tumor antigen binding sites I and II. On the late strand that is the template for late mRNA synthesis, six major initiation sites were observed, each within the 3′ C-C-C-G-C-C 5′ sequence in the template that is repeated twice within each of the three 21-bp repeats that lie adjacent to the minimal origin, on its late side. A 6-bp deletion in the 65-bp minimal origin that eliminates its function as an origin reduced the major initiations around nucleotide 5215 on the early strand by 90% but did not affect initiations at the minor start site on the early strand or initiations on the late strand. Mouse DNA primase is able to recognize specific regions on the SV40 DNA. Those on the early strand are within the minimal origin of replication and those on the late strand are within the 21-bp repeat region necessary for maximum replication.**

DNA primase is a generic name for a RNA polymerase that synthesizes oligoribonucleotides that prime the initiation of DNA synthesis (1). In eukaryotes, RNA primers of Okazaki fragments have been characterized as oligoribonucleotides of 8–10 nucleotides (2–4), the length of which remains relatively invariant upon perturbation of nucleotide concentrations (5, 6). These characteristics have allowed the identification and purification of a DNA primase from mouse hybridoma cells (7) and *Drosophila melanogaster* (8, 9) and reports of similar activities from a number of other eukaryotic sources (10–15).

Primer RNAs were characterized by examination of discontinuous DNA synthesis. The role of RNA priming or of DNA primase at an origin of replication in eukaryotic cells is less clear. Simian virus 40 (SV40) DNA replication is a model of a single replicon and is well-defined as to its origin of replication. A 65-base-pair (bp) region is a minimal regulatory sequence required for (*i*) DNA synthesis determined by viable viral mutants (16) and (*ii*) the capacity to act as a replication element in *cis* when supplied with tumor (T) antigen (17, 18). The origin of replication may extend from the 65-bp minimal sequence 10 nucleotides to the early side, as some deletions in this area reduce DNA synthesis (16, 17), and ≈50 bp to late side, as removal of all three 21-bp repeats also decreases the ability to replicate (18). The 65-bp region may be considered an essential *cis*-acting element for replication, whereas adjacent regions to the early and late side are required for maximum replication.

In this work, DNA primase from mouse hybridoma cells (7) was used to examine sites of initiation within a segment of SV40 DNA that includes the origin of replication.

MATERIALS AND METHODS

Materials. Mouse primase and DNA polymerase α were purified as described (7). DNA polymerase I large fragment was purchased from Boehringer Mannheim. Nucleotides were from P-L Biochemicals, and radioactive nucleotides were from Amersham. Restriction enzymes were from New England BioLabs or Bethesda Research Laboratories. *Escherichia coli* JM101 and M13 mp7 were obtained from Bethesda Research Laboratories.

Primase Initiation Reactions. Standard reactions were carried out in 20 μl of medium containing 50 mM potassium Hepes (pH 7.6), 10 mM $MgCl_2$, 0.5 mM rATP, 0.1 mM rCTP, 0.1 mM rGTP, 0.1 mM rUTP, 0.01 mM dNTP, [α-^{32}P]dATP (2.2×10^4 cpm/pmol), 1 mM dithiothreitol, 25 mM KCl (from enzyme addition), bovine serum albumin (50 μg/ml), DNA template (A_{260} = 0.1), mouse primase (10 units), and DNA polymerase (0.8 unit). After 60 min at 30°C, *E. coli* DNA polymerase I large fragment (0.01 unit) was added along with unlabeled dNTP in 50-fold excess, and incubation was continued at room temperature for 30 min. Reactions were terminated by addition of 80 μl of 10 mM Tris·HCl, pH 8.1/20 mM EDTA/0.2% $NaDodSO_4$/0.5 mg of yeast RNA per ml, and nucleic acids were precipitated with 0.1 ml of 1 M KCl and 0.6 ml of ethanol. Precipitates were collected, washed with 70% ethanol, and dried. Samples were resuspended in 50% dimethyl sulfoxide/10 mM Tris borate, pH 8.3/0.2 mM EDTA, and a portion was electrophoresed on a 6% polyacrylamide gel (46 × 19 × 0.03 cm) in 7 M urea/50 mM Tris borate, pH 8.3/1 mM EDTA.

Template Cloning and Isolation. SV40 *Eco*RII G fragments, wild type and DL1, were isolated from pSV01 and pDL1 (ref. 17; gift of R. Tjian), plasmids that contained the *Eco*RII G fragment with *Eco*RI linkers. The plasmids were cut with *Eco*RI nuclease, the insert was isolated by agarose gel electrophoresis, and the ends were filled by using *E. coli* DNA polymerase I large fragment. The blunt-end fragments were mixed with M13 mp7 replicative form that had been digested with *Hin*cII and bacterial alkaline phosphatase and ligated with T4 DNA ligase according to manufacturers' specifications. *E. coli* JM101 cells were transformed with the reaction mixture. Recombinant phages were selected, and their orientation was determined by annealing. Two phages with opposite orientations of the insert were selected. After growth, phage was concentrated by precipitation with 3% polyethylene glycol 6000/0.5 M NaCl. DNA was released from the phage by treatment with 2% $NaDodSO_4$, extracted

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Abbreviations: bp, base pairs; SV40, simian virus 40; T antigen, tumor antigen.

with phenol, and precipitated with ethanol. The DNA was resuspended, and the solution was adjusted to 6% polyethylene glycol 6000/0.5 M NaCl and held at room temperature for 1 hr; the precipitate that formed was removed by centrifugation. The phage DNA remained in the supernatant and was precipitated with 2 vol of ethanol or addition of polyethylene glycol to 10%.

Cloning into M13 mp7 vector allowed the isolation of the cloned insert as single-stranded DNA from the phage DNA because of the symmetry of restriction enzyme sites in the cloning region of M13 mp7 (19). For isolation of the single-stranded insert, phage DNA was incubated at 60°C for 5 min and then at 45°C for 5 min and was allowed to cool to room temperature (to promote formation of double-stranded regions) before digestion in 25 mM potassium Hepes, pH 7.6/7 mM $MgCl_2$/100 mM NaCl/1 mM dithiothreitol containing 0.5 mg of DNA and 250 units of *Bam*HI restriction nuclease per ml at 37°C for 16 hr. Samples were extracted with phenol and precipitated with ethanol. The short insert was isolated by size fractionation with 8% polyethylene glycol 6000/0.5 M NaCl that precipitates the majority of the large vector DNA and then by sedimentation through a 5–20% sucrose gradient in 10 mM Tris·HCl, pH 7.6/0.1 M NaCl/1 mM EDTA in a SW60 rotor (Beckman) at 45,000 rpm for 5 hr at 20°C. Fractions were collected and monitored by gel electrophoresis, and the insert DNA was pooled and collected by ethanol precipitation.

Sequence of Templates. Single-stranded DNA for sequence determination was labeled at the 3′ end by fill-in of the *Bam*HI site on the single-stranded insert using [α-^{32}P]dCTP, dNTP, and *E. coli* DNA polymerase I large fragment and was chemically degraded as described by Maxam and Gilbert (20). The four chemical reactions cleaved at G, A+G, C+T, and C nucleotides. The sequence of the single-stranded fragments was determined by both 3′ labeling and 5′ labeling to be

5′ G-A-T-C-C-G-T-C-A-A-T-T-C-C———SV40 *Eco*RII G fragment early or late strand

3′ G-C-A-G-T-T-A-A-G-G———

and contained, in addition to the SV40 sequences, DNA sequences from a part of the *Eco*RI linkers and M13 mp7 cloning site. The SV40 *Eco*RII G fragment sequence (5′ to 3′) for the early strand is nucleotides 5097–159 and for the late strand is nucleotides 159–5097. The *Eco*RII recognition sites had been removed in the initial construction of pSV01 (17). The 3′-^{32}P-labeled late-strand sequence ladder was compared with the products of primase incubations with the early-strand template and the converse. Nucleotide positions were as described by Van Heuverswyn and Fiers (21).

RESULTS

Mapping Procedure. To determine the nucleotide position of initiation by primase, RNA primers were extended with DNA to the end of the template by DNA polymerases with labeled dNTPs, and the migration positions of the products were compared to a sequence ladder produced by chemical degradation (20) of 3′-labeled [^{32}P]DNA of the same sequence. However, the chain length is one nucleotide shorter because the chemical degradation procedures remove the nucleoside. The nucleotide positions of initiation sites are reported with this subtraction. In addition, a 5′ triphosphate on the primer contributes additional charge (−2) that may affect migration dependent upon chain length; a similar charge from a terminal phosphate does not affect migration of chains longer than 50 nucleotides (22). No compensation was made for the extra phosphates or potential differences in migration due to the RNA of the primer.

Primase Initiation on the Early Strand of SV40 That Includes the Origin of Replication. The *Eco*RII G fragment (311 bp) of SV40 that contains the origin of replication was cloned into the M13 mp7 vector, and the single-stranded DNA insert was isolated from the phage DNA after restriction nuclease cleavage as described. The early strand of the *Eco*RII G fragment, which is the template for early mRNA synthesis, was used as template in an incubation along with mouse primase and DNA polymerase α. The labeled DNA products were separated according to chain length by polyacrylamide gel electrophoresis (Fig. 1*A*, lane 1). Labeled DNAs up to ≈130 nucleotides long were synthesized that migrated around nucleotide 5220 and below. DNA polymerase α is retarded by secondary structures (23, 24); to extend the chains to the end of the template, *E. coli* polymerase I large fragment was added along with excess unlabeled dNTP. Nearly all of the label that appeared at various chain lengths was extended and found to initiate from four positions within 10 nucleotides of each other around nucleotide 5215 (Fig. 1*A*, lanes 2 and 3). An additional group of start sites was observed at nucleotides 5145 and 5147 (Fig. 1*A*, lane 2) but was present at about one-sixth the level of those around nucleotide 5215, when one takes into account the shorter chain length. No other initiations were observed on the early strand for the regions examined (nucleotides 5117–5243/0–140). Primase and rNTPs were required for initiation.

The positions of the major starts (Fig. 1*A Inset*, lane 3) indicates that the initiating nucleotides would be guanosine (5211), uridine (5215), guanosine (5219), and cytidine (5221). However, the chains were labeled with [γ-^{32}P]rATP but not [γ-^{32}P]rGTP (not shown). The positions that are consistent with adenosine initiation and with the observed spacing between starts are one nucleotide shorter at nucleotides 5210, 5214, 5218, and 5220. Therefore, the nucleotide positions of initiation are tentative but would not appear to be misplaced by more than one nucleotide. It is unclear why there may be a difference in migration between product and sequence ladder, but possibilities are that the 5′ triphosphate group or RNA primer affect migration.

To demonstrate that the DNA chains were completed to the end of the template, the products were digested with restriction nuclease *Sau*96I (nucleotide 5121) that cleaves 39 nucleotides from the end of the template. The chain lengths of all four initiations around nucleotide 5215 were reduced in length by 39 nucleotides relative to the sequence ladder. In addition, cleavage with restriction nuclease *Hae* III (nucleotide 5192) should result in chain lengths of 18, 22, 26, and 28 nucleotides from the four initiation sites (around nucleotide 5215) to the *Hae* III site. The observed products were 20, 23.5, 27.5, and 29.5 nucleotides long relative to the sequence ladder. This again indicates that the tentative map positions are within one or two nucleotides of the actual start.

Mouse primase synthesized RNA in multiples of 10 nucleotides. When DNA polymerase α was present, only primers ≈10 nucleotides long were synthesized (7, 25). To determine the size of the RNA primers synthesized on the early strand, labeled DNA products from each major initiation site were isolated, and the RNA was removed by alkaline hydrolysis. The DNA chain lengths were shortened by 8–10 nucleotides after removal of the RNA (Fig. 2), and their positions were at nucleotides 5200–5213. Each initiation site showed several positions for the transition of RNA to DNA. This indicates that the initiation site was specific but that the chain length

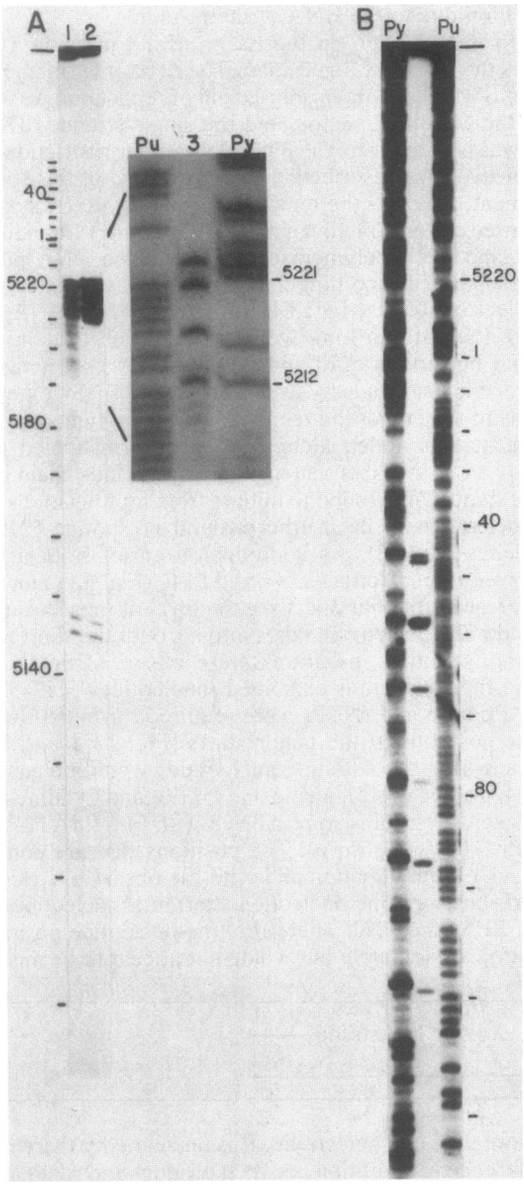

FIG. 1. Primase initiation on single-stranded DNA of SV40 *Eco*RII G fragment. (*A*) The early strand of *Eco*RII G fragment was incubated with primase and DNA polymerase α (lane 1), followed by *E. coli* DNA polymerase I large fragment (lane 2) as described, and the products were separated by polyacrylamide gel electrophoresis. (*Inset*) Initiation sites at nucleotide resolution along with purine (Pu) and pyrimidine (Py) sequence assay reactions of 3′-^{32}P-labeled *Eco*RII G late strand. (*B*) The late strand of *Eco*RII G fragment was incubated under standard conditions and fractionated by polyacrylamide gel electrophoresis. The sequence assay reactions of purine and pyrimidines of the 3′-^{32}P-labeled *Eco*RII G early strand are shown. The top line indicates the migration position of intact template strands that are also labeled. The nucleotide positions shown alongside the figures are taken from a sequence ladder, and the positions are numbered as described by Van Heuverswyn and Fiers (21).

of the RNA primer can vary by one or two nucleotides before the start of DNA synthesis.

To assess the ability of primase (without DNA polymerase α) to synthesize specific transcripts for initiation, templates were preprimed with primase, enzyme was removed by phenol extraction, and the RNA-primed template was incubated sequentially with DNA polymerase α and *E. coli* DNA polymerase I large fragment, as before. Identical initiation sites were observed for incubation with preprimed templates. Only primase, then, is required for site-specific initiations.

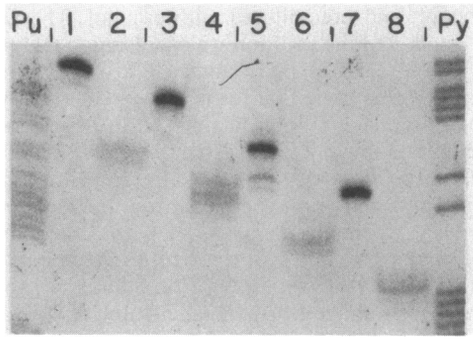

FIG. 2. Alkaline hydrolysis of primase-initiated DNA. A standard reaction was carried out with the early-strand template, and the products were separated by polyacrylamide gel electrophoresis as shown in Fig. 1*A*. The products at each initiation site were eluted from the gel in 2 M LiCl/0.2% $NaDodSO_4$/10 mM Tris·HCl, pH 7.6/1 mM EDTA and were recovered by precipitation with ethanol. Samples from nucleotide positions 5220 (lanes 1 and 2), 5218 (lanes 3 and 4), 5214 (lanes 5 and 6), and 5210 (lanes 7 and 8) were divided, and half of each sample was incubated in 10% piperidine at 37°C for 16 hr. The untreated (lanes 1, 3, 5, and 7) and alkaline hydrolyzed (lanes 2, 4, 6, and 8) samples were dried and fractionated on a 6% polyacrylamide gel. The sequence ladders are purine (Pu) and pyrimidine (Py) reactions of 3′-^{32}P-labeled late strand.

In support of this, the same initiation sites were observed in incubations with primase and *E. coli* DNA polymerase I or avian myeloblastosis virus reverse transcriptase, although at lower levels than when DNA polymerase α was present.

Major primase initiations on the early strand of *Eco*RII G fragment are within the 65-bp minimal origin of replication region and between T-antigen binding sites I and II.

Initiation Sites on the Late Strand of *Eco*RII G Fragment. Primase initiation sites were mapped on the late strand of *Eco*RII G fragment by the same procedures. Six major sites were found (Fig. 1*B*), each of which was within the six repeats of 3′ C-C-C-G-C-C 5′ in the template that are part of the three 21-bp repeats to the late side of the origin. Several other minor starts also were observed to lie within the 21-bp repeats and a very weak site in the A+T-rich region of the minimal origin (nucleotide 21).

The major initiation sites on the late strand did not migrate in phase with the sequence ladder and are tentatively assigned to nucleotides 47 and 48, 58, 68, 80, 89, and 101. These are tentative assignments for the reasons given for the early-strand initiations. These positions correspond to the Cs on the 5′ side of G in the template repeat 3′ C-C-C-G-C-C 5′, and the products are labeled with [γ-^{32}P]rGTP (not shown).

Digestion with restriction nuclease *Sph* I (nucleotide 132) shortened all chains by the expected amount, and all initiations were primed with an RNA $\approx$10 nucleotides long.

The number of initiations at each site for both early and late strand was calculated from incorporated [^{32}P]dAMP and corrected for dAMP content in each chain from the DNA sequence (Fig. 3*A*). Initiation from each of the major sites on the early strand occurred with equal frequency and normalized to 1. The starts on the late-strand template differed by factors of 1–6. The two strongest initiation sites were on the late strand in the 21-bp repeat that is closest to the minimal origin, that is part of T-antigen binding site III, and that is an imprecise copy of the other two 21-bp repeats.

Primase Initiation on DL1 Template That Is Defective in Replication. Myers and Tjian have constructed deletion mutants within the *Eco*RII G fragment to study T-antigen binding and DNA replication (17). One of these, DL1, has a 6-bp deletion within T-antigen binding site II near the middle of the 65-bp minimal origin, which eliminated the ability to rep-

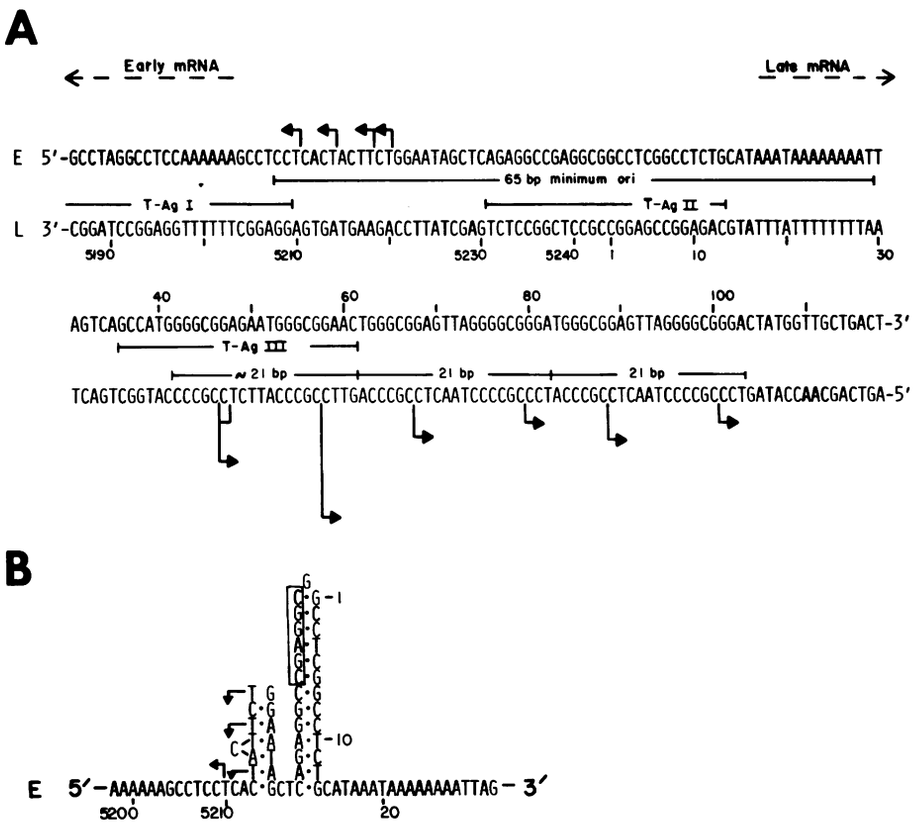

FIG. 3. Summary of primase initiation sites in the SV40 origin region. (*A*) The nucleotide sequence of a portion of the *Eco*RII G fragment is shown along with primase initiation sites. The sites are tentative and are indicated between two possible nucleotides. The initiation sites on the early strand are most consistent with nucleotides 5210, 5214, 5218, and 5220, as described in the text. The height of the starts corresponds to the frequency of initiation from that site, and the arrows indicate the direction of synthesis. Also shown are the 65-bp minimal origin of replication (16), T-antigen binding sites (26), the three 21-bp repeats, and the direction of early and late mRNA synthesis. The nucleotide positions are numbered as described by Van Heuverswyn and Fiers (21). (*B*) Potential secondary structure near the primase initiation sites (arrows) on the early strand. The boxed sequence is deleted in the DL1 mutant.

licate when provided with T antigen but bound T antigen because site I is the strongest binding site (17, 26). The *Eco*RII G fragment with the DL1 deletion was recloned in M13 mp7, and the single-stranded DNA inserts in both orientations were purified after excision. In parallel incubations with the early strand of *Eco*RII G wild-type and DL1 fragments, primase initiations from the major site (around nucleotide 5215) were reduced by ≈90% with the DL1 template in comparison to wild type (Fig. 4, lanes 1 and 2), whereas the frequency of initiation from the minor site (around nucleotide 5147) was unchanged. With the late-strand templates, the frequency of initiations showed no significant change for any of the sites (Fig. 4, lanes 3 and 4).

The DL1 mutant, we find, has 6 bp deleted (nucleotides 5237–5242) around the *Bgl* I site rather than 4 bp (17). The deletion lies at the closest 16 nucleotides upstream from the four major initiation sites on the early strand, but secondary structure may bring the region closer because it is part of the stem, which is a 27-nucleotide perfect inverted repeat (Fig. 3*B*). This structure may play a role, then, in site recognition by primase on the early strand.

DISCUSSION

We examined the ability of purified mouse primase (7) to initiate DNA synthesis on single-stranded templates from the origin region of SV40 and found that primase showed strong site specificity. Of a total of ≈600 nucleotides examined for the two strands of the *Eco*RII G fragment, two regions, one on each strand, were the major sites for primase initiation of DNA synthesis. With the early-strand template, the major initiation site consisted of four start points within 10 nucleotides of each other, tentatively assigned nucleotides 5210, 5214, 5218, and 5220. These initiation sites appear to be significant, as they are the only primase sites within the SV40 65-bp minimal origin of replication (Fig. 3*A*), and a deletion mutant that is unable to replicate (17) reduced initiations by 90% from these sites only (Fig. 4).

On the late strand, primase initiations occurred in the 21-bp repeat region within each of the six repeats of 3′ C-C-C-G-C-C 5′ that comprise part of the three 21-bp repeats. The two strongest primase starts were in the 21-bp repeat that is closest to the origin. This repeat contains T-antigen binding site III and is not an exact copy of the other two repeats (Fig. 3*A*). These sequence differences may enhance primase initiations. Preliminary experiments (unpublished data) show that in addition to the aforementioned start sites on the 5′ side of G in the six repeats of 3′ C-C-C-G-C-C 5′, additional sites are used on the 3′ side of G when the rGTP concentration is increased. The 21-bp repeats are apparently designed to be redundant for primase initiations with >15 start sites within ≈60 nucleotides. In studies of the function of the 21-bp repeats, the two repeats closest to the origin were required for maximum replication, and, in the absence of both, replication was reduced by 80% (18). However, the repeat region is not sufficient without the minimal origin (16–18), which would suggest that the first events of DNA synthesis are toward the early side. In addition, primase starts that are in the 21-bp repeat region suggest a possible mechanism for stimulating replication by facilitating formation of a replication fork toward the late side.

All of the initiations that we observe with primase are for leading strand synthesis away from the origin. Consistent

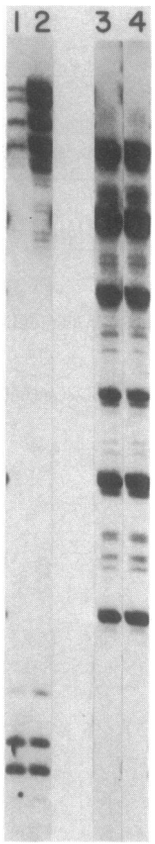

FIG. 4. Primase starts in *Eco*RII G fragment of wild-type and DL1 mutant templates. Standard primase initiation reactions were carried out with *Eco*RII G early strands of DL1 (lane 1) and wild type (lane 2) and with *Eco*RII G late strands of DL1 (lane 3) and wild type (lane 4). The products were fractionated by polyacrylamide gel electrophoresis.

with this is the location of the transition of continuous–discontinuous DNA synthesis of RNA–DNA junctions in SV40 replicative intermediates at nucleotides 5210–5211 (27). The RNA–DNA junctions for early-strand primase initiations overlap this region (nucleotide 5200–5213). However, primase initiations on the late strand in this region were not observed, nor were initiations for lagging strand synthesis. This may reflect possibilities, such as a need for additional factors as in a replication complex, a lower efficiency of initiation for the discontinuous strand, or a requirement for another DNA primase for initiation of discontinuous synthesis. Mouse primase, which we have used here, does not exhibit absolute specificity for SV40 origin sequences. A variety of DNA templates, both synthetic and natural, were utilized (7, 25); upon increasing the detection sensitivity of primase initiation 10-fold, additional sites are seen on SV40 *Eco*RII G fragment DNA, indicating that other sites can be used but at much lower frequencies. However, a primase activity was reported to copurify with DNA polymerase α from a variety of sources (8, 10, 14, 15), a property we have not observed for either primase or DNA polymerase α from mouse hybridoma cells (7). In the case of *Drosophila melanogaster* polymerase–primase, primase activity is associated with subunits (9) similar in size to mouse primase (7), suggesting conserved activities. It remains to be shown, then, whether there are different primase activities in the cell.

As the nucleotide positions are tentative, we have not examined the initiation sites for consensus sequences, but a sequence in the template of 3′ Y_n-R-Y_n 5′, where $n \geq 2$ and Y and R are unspecified pyrimidine and purine nucleosides, is common in the late strand to the 3′ C-C-C-G-C-C 5′ repeats and in the early strand to the minor start and part of the major starts, although not all such regions in the template are used.

The specific initiation by primase in the origin of replication region of SV40 strongly indicates that specificity of DNA initiation rests at least at two levels—T-antigen interaction with the origin region and site-specific initiation by primase after strand separation. The experiments presented suggest that the initiation of SV40 DNA synthesis can be defined at the nucleotide level and will allow study of the function of T-antigen in DNA replication. It also would be of interest to compare primase initiations in the origin region of SV40 with other papovaviruses and to determine its usefulness in characterizing cellular origins of replication.

Note Added in Proof. We have confirmed the major initiation sites on the early strand to be at nucleotide positions 5210, 5214, 5218, and 5220 from a partial sequence determined by primase incorporation of [γ-^{32}P]rATP or [γ-^{32}P]rGTP at major and minor initiation sites in that region.

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1. Kornberg, A. (1980) *DNA Replication* (Freeman, San Francisco).
2. Reichard, P., Eliasson, R. & Söderman, G. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 4901–4905.
3. Tseng, B. Y. & Goulian, M. (1977) *Cell* **12**, 483–489.
4. Kaufmann, G., Anderson, S. & DePamphilis, M. (1977) *J. Mol. Biol.* **116**, 549–567.
5. Eliasson, R. & Reichard, P. (1978) *Nature (London)* **272**, 184–185.
6. Tseng, B. Y. & Goulian, M. (1980) *J. Biol. Chem.* **255**, 2062–2066.
7. Tseng, B. Y. & Ahlem, C. N. (1983) *J. Biol. Chem.* **258**, 9845–9849.
8. Conaway, R. C. & Lehman, I. R. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 2523–2527.
9. Kaguni, L. S., Rossignol, J.-M., Conaway, R. C., Banks, G. R. & Lehman, I. R. (1983) *J. Biol. Chem.* **258**, 9037–9039.
10. Yagura, T., Kozu, T. & Seno, T. (1982) *J. Biol. Chem.* **257**, 11121–11127.
11. Tseng, B. Y. & Ahlem, C. N. (1982) *J. Biol. Chem.* **257**, 7280–7283.
12. Kaufmann, G. & Falk, H. H. (1982) *Nucleic Acids Res.* **10**, 2309–2321.
13. Méchali, M. & Harland, R. M. (1982) *Cell* **30**, 93–101.
14. Shioda, M., Nelson, E. M., Bayne, M. L. & Benbow, R. M. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 7209–7213.
15. Hubscher, U. (1983) *EMBO J.* **2**, 133–136.
16. DiMaio, D. & Nathans, D. (1980) *J. Mol. Biol.* **140**, 129–142.
17. Myers, R. M. & Tjian, R. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 6491–6495.
18. Bergsma, D. J., Olive, D. M., Hartzell, S. W. & Subramanian, K. N. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 381–385.
19. Patton, J. R. & Chae, C.-B. (1982) *Anal. Biochem.* **126**, 231–234.
20. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65**, 499–560.
21. Van Heuverswyn, H. & Fiers, W. (1979) *Eur. J. Biochem.* **100**, 51–60.
22. Tapper, D. P. & Clayton, D. A. (1981) *Nucleic Acids Res.* **9**, 6787–6794.
23. Weaver, D. T. & DePamphilis, M. L. (1982) *J. Biol. Chem.* **257**, 2075–2086.
24. Kaguni, L. S. & Clayton, D. A. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 983–987.
25. Tseng, B. Y. & Ahlem, C. N. (1984) in *Mechanisms of DNA Replication and Repair*, ed. Cozzarelli, N. (Liss, New York), pp. 511–516.
26. Tjian, R. (1979) *Cold Spring Harbor Symp. Quant. Biol.* **43**, 483–489.
27. Hay, R. T. & DePamphilis, M. L. (1982) *Cell* **28**, 767–779.