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DNA binding site for a factor(s) required to initiate simian virus 40 DNA replication

(simian virus 40 large tumor antigen/eukaryotic DNA replication/replication origin)

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ABSTRACT **Efficient initiation of DNA replication in the absence of nonspecific DNA repair synthesis was obtained by using a modification of the system developed by J. J. Li and T. J. Kelly [(1984) *Proc. Natl. Acad. Sci. USA* 81, 6973–6977]. Circular double-stranded DNA plasmids replicated in extracts of CV-1 cells only when the plasmids contained the *cis*-acting origin sequence for simian virus 40 DNA replication (*ori*) and the extract contained simian virus 40 large tumor antigen. Competition between plasmids containing *ori* and plasmids carrying deletions in and about *ori* served to identify a sequence that binds the rate-limiting factor(s) required to initiate DNA replication. The minimum binding site (nucleotides 72–5243) encompassed one-half of the simian virus 40 *ori* sequence that is required for initiation of replication (*ori*-core) plus the contiguous sequence on the late gene side of *ori*-core containing G+C-rich repeats that facilitates initiation (*ori*-auxiliary). This initiation factor binding site was specific for the simian virus 40 *ori* region, even though it excluded the high-affinity large tumor antigen DNA binding sites.**

With the exception of initiation of DNA replication, all subsequent steps in the replication and assembly of simian virus 40 (SV40) chromosomes utilize host-cell components exclusively (1, 2). Initiation of viral DNA replication requires the *cis*-acting 63-base-pair (bp) *ori*-core sequence, SV40 large tumor antigen (T-Ag), and permissive cell factors from monkey or human cells. Bidirectional replication then originates at the junction between the strongest DNA binding site for T-Ag and *ori*-core (Fig. 1). The 40–45 bp flanking either side of *ori*-core (*ori*-auxiliary; Fig. 1) are not required for replication, although they do stimulate replication 3- to 5-fold.

Purified SV40 T-Ag binds most strongly to the early gene-side auxiliary sequence (site I), less strongly to the *ori*-core sequence (site II), and marginally to the late gene-side auxiliary sequence and beyond (site III; Fig. 1). Mutations within sites I and II alter the affinity of T-Ag for DNA (6–8, 19). These sites are biologically significant because mutations within *ori* that reduce or prevent replication can be suppressed by mutations in T-Ag (20), and mutations within site I that reduce the ability of T-Ag to regulate its own synthesis are overcome by mutations in T-Ag (7). Since most of binding sites I and III can be deleted with only a marginal effect on replication, initiation of DNA replication must involve binding of T-Ag to site II (*ori*-core), perhaps aided by the strong affinity of T-Ag to site I. Thus, one might expect *ori*-core and perhaps T-Ag binding site I to be the primary binding site for the protein complex responsible for initiating SV40 DNA replication.

With the advent of soluble systems capable of initiating SV40 DNA replication *in vitro* (21–24), it was possible to determine which DNA sequence exhibited the strongest affinity for replication initiation factors by its ability to inhibit replication of a second plasmid containing the SV40 *ori*-region. Surprisingly, the DNA binding site for a limiting factor(s) required to initiate SV40 DNA replication did not coincide with *ori*-core and did not include the strongest T-Ag binding sequences, although it was specific for SV40.

MATERIALS AND METHODS

Cellular extracts were prepared and DNA replication was carried out under conditions similar to those described by Li and Kelly (23), but with modifications that improved the system's efficiency. A detailed description and characterization will be published elsewhere. CV-1 cells were infected with SV40 wt800 (13), and 36 hr later they were extracted with 20 mM Hepes, pH 7.8/5 mM potassium acetate/0.5 mM $MgCl_2$/0.5 mM dithiothreitol (low-salt extract; see ref. 25). Nuclei were extracted a second time for 1 hr at 0°C with the same solution adjusted to 0.5 M potassium acetate (high-salt nuclear extract). The DNA replication assay (50 μl) contained 42 mM Hepes (pH 7.8), 102 mM potassium acetate, 7 mM Mg acetate, 1 mM EGTA, 0.8 mM dithiothreitol, 4 mM ATP, 0.2 mM each of CTP, UTP, and GTP, 0.1 mM each of dATP and dGTP, 40 μM each of [α-^{32}P]dTTP and [α-^{32}P]dCTP (5–10 μCi; 1 Ci = 37 GBq), 10 mM phospho*enol*pyruvate, 3 μg of phospho*enol*pyruvate kinase, 0.5 μg of DNA, 20 μl of low-salt extract (110–150 μg of protein), 10 μl of high-salt nuclear extract (60–80 μg of protein), and 3–6% polyethylene glycol (M_r, 14,000). Reaction mixtures were incubated 2.5 hr at 30°C and then adjusted to 0.5% sodium dodecyl sulfate/15 mM EDTA/tRNA (200 μg/ml)/proteinase K (100 μg/ml), and incubated 30 min at 37°C. DNA was purified, and one aliquot was digested with a 3- to 5-fold excess of restriction enzymes under conditions specified by the suppliers. DNA samples were fractionated by electrophoresis in 0.6% agarose gels and ^{32}P-labeled DNA bands were visualized by autoradiography (26, 27). SV40 T-Ag was purified from Ad5SVRIII-infected human 293 cells by immunoaffinity chromatography using monoclonal antibody pAb419 (28).

RESULTS

SV40 *ori*-Dependent DNA Replication *in Vitro*. Various plasmid DNAs were tested for their ability to replicate when incubated in an extract of SV40-infected CV-1 cells similar to the one previously shown by Li and Kelly (23, 24) to support normal SV40 DNA replication. DNA synthesis was detected by incorporation of [α-^{32}P]dNTPs. DNA replication was measured by the conversion of DNA that was sensitive to

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Abbreviations: SV40, simian virus 40; bp, base pair(s); T-Ag, large tumor antigen; kb, kilobase(s).

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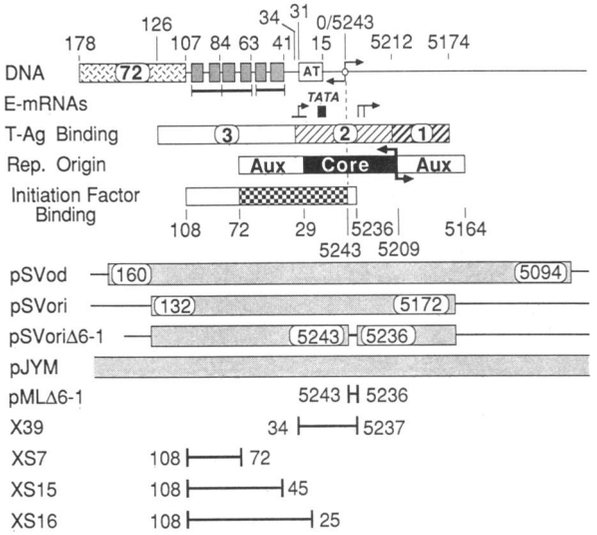

FIG. 1. Plasmids containing deletions in and around the SV40 *ori* region. Relevant SV40 DNA sequences are a 27-bp palindrome ( ), A+T-rich region (AT), six $G_{3-4}CG_2Pu_2$ repeats (shaded blocks), three 21-bp repeats (solid lines), and two 72-bp repeats (only one is shown; mottled block). The G+C-rich region and 72-bp repeats contain promoter and enhancer elements (1, 2). DNA binding sites for T-Ag (2, 3–6) are shaded according to their affinity (1 > 2 > 3). *Ori* (Rep. Origin) consists of a required sequence (Core, 29–5209) flanked by two auxiliary sequences (Aux) (2, 7–11) with the transition point between continuous and discontinuous DNA synthesis (i.e., origin of bidirectional replication) designated by arrows (12, 13). The primary binding site for a replication factor(s) required to initiate SV40 DNA replication is indicated with the minimum required sequence shaded (Initiation Factor Binding). Plasmids containing SV40 sequences are represented by a shaded bar for SV40 DNA and a line for plasmid DNA. pJYM (8212 bp; ref. 14) and pMLΔ6-1 (15) contain the entire SV40 genome cloned into pML-1, except that pMLΔ6-1 has a 6-bp deletion within SV40 *ori*. pML-1 (2969 bp) is a pBR322 plasmid minus sequences that poison DNA replication in mammalian cells (14). pSVod (3300 bp) contains the 311-bp *Bst*N1 SV40 DNA fragment cloned into a 3-kilobase (kb) segment of pBR322 DNA (16). pSVori (2643 bp) and pSVoriΔ6-1 were constructed by inserting the 206-bp *Hin*dIII/*Sph* I SV40 DNA fragment from pJYM or pMLΔ6-1 between the *Hin*dIII and *Sph* I sites of pML-1. Plasmids X39, XS7, XS15, and XS16 (7451–7498 bp) consist of the SV40 genome cloned into a 2.3-kb segment of pBR322 DNA and contain deletions within and around SV40 *ori* as indicated (17). Terminal nucleotide positions for SV40 strain 777 sequences are indicated. All plasmids were propagated in *Escherichia coli* HB101 (dam$^+$) and their DNA was purified as described by Maniatis *et al.* (18).

cleavage with *Dpn* I restriction endonuclease to DNA that was resistant to cleavage. Low molecular weight DNA purified from these incubations was first digested with *Eco*RI to convert all forms of monomeric DNA into linear molecules (form III) that would migrate as a single band during gel electrophoresis. Each plasmid contained from 17 (pML-1) to 25 (pJYM, pB1a) *Dpn* I sites that were methylated by growth in a dam$^+$ *Escherichia coli* strain and thus rendered sensitive to cleavage by *Dpn* I. Since mammalian cells do not contain this methylase, DNA replication *in vitro* produced hemimethylated and unmethylated DNA, both of which were insensitive to cleavage by *Dpn* I (29). Therefore, the total amount of form III ^{32}P-labeled plasmid DNA observed was a measurement of total *in vitro* DNA synthesis (i.e., replication plus repair), while the amount of *Dpn* I resistant form III ^{32}P-labeled plasmid DNA measured only DNA replication. When cut with *Eco*RI, replicating DNA intermediates (RI) produced molecules with branches of various lengths, depending on the extent of their replication, that migrated more slowly than form III DNA during gel electrophoresis and appeared as a smear of radioactivity (30, 31; Fig. 2).

As previously reported (23, 24), plasmids containing the SV40 *ori* region such as pSVori, pSVod, and pJYM (Fig. 1) replicated (Fig. 2, lanes g, j, m, and n; Fig. 3, lane b′; data not shown). Deletions of the late gene side *ori*-auxiliary sequence (XS7, XS15; Fig. 1) also replicated (Fig. 3, lanes y and z). In contrast, the same plasmid without the SV40 *ori* region (pML-1) or containing a nonfunctional *ori*-core region such as pSVoriΔ6-1, pMLΔ6-1, X39, and XS16 (Fig. 1) failed to replicate (Fig. 2, lanes h, k, and q; Fig. 3, lanes v, x, and a′; data not shown). Therefore, *ori*-core was required for initiation of replication. Furthermore, DNA replication was specific for the SV40 *ori* region. pML-1 containing the polyoma virus *ori* region (pPyVori), a complete polyoma virus genome (pB1a), or 17 tandemly joined *Xho* I linkers (pXhoI) failed to replicate (Fig. 2, lanes o and p; data not shown). Tandemly arranged *Xho* I linkers generate a repeating GAGGC sequence that binds tightly to either SV40 or polyoma virus T-Ag (33).

We observed that a high-salt nuclear extract stimulated DNA replication in this system ≈50% (Fig. 2, compare lanes a and d, g and j), eliminated the small amount of repair synthesis (*Dpn* I sensitive) seen with pSVoriΔ6-1 (compare lanes b and e), and allowed synthesis of covalently closed superhelical (form I) DNA (data not shown). One function of nuclear extract was to provide T-Ag. pSVori replicated poorly when incubated with SV40 chromatin that had been isolated by sedimentation (29) and resuspended in a low-salt extract from uninfected CV-1 cells (lane v). However, addition of either nuclear extract from SV40-infected CV-1 cells (lane r) or purified SV40 T-Ag (lane x) resulted in an 8- to 9-fold increase in DNA replication, while addition of both nuclear extract and purified T-Ag stimulated DNA replication 12-fold (lane t). Addition of T-Ag was required absolutely when both extracts were prepared from uninfected CV-1 cells (data not shown). pSVoriΔ6-1 failed to replicate under any of these conditions (lanes s, u, w, and y). Therefore, DNA replication in this system required both SV40 *ori* and T-Ag.

DNA synthesis was linear for at least 3 hr. Digestion with appropriate restriction endonucleases of newly replicated DNA that had been radiolabeled for various periods of time revealed that DNA replication was bidirectional from SV40 *ori* (unpublished results). Furthermore, ≈30% of the newly replicated DNA was not only resistant to cleavage by *Dpn* I, but was digested completely by *Mbo* I. Since *Mbo* I cuts DNA at the same sites recognized by *Dpn* I, but only if neither strand is methylated, this newly replicated DNA must have undergone two or more rounds of replication. Replication was sensitive to aphidicolin, a specific inhibitor of DNA polymerase α, and resistant to α-amanitin at 250 μg/ml, a specific inhibitor of RNA polymerases II and III.

Interference of SV40 *ori*-Dependent DNA Replication by Specific DNA Sequences. The DNA binding site for factors required to initiate SV40 DNA replication was identified by its ability to interfere with pSVod replication when added to the *in vitro* system as a double-stranded circular plasmid DNA. The competitor DNA should have interfered with pSVod replication only if it adsorbed one or more factors from the reaction that were required for replication. In this way, the DNA binding site for the factor(s) present in lowest concentration could be identified, since the availability of this factor would be the rate limiting step in replication.

Only DNA molecules containing an active SV40 *ori* region interfered strongly with the replication of pSVod. For example, addition of pSVori to an *in vitro* DNA replication system containing pSVod sharply reduced the amount of *Dpn* I-resistant pSVod form III ^{32}P-labeled DNA produced (Fig. 3, lanes d–f), a result that was similar to increasing the concen-

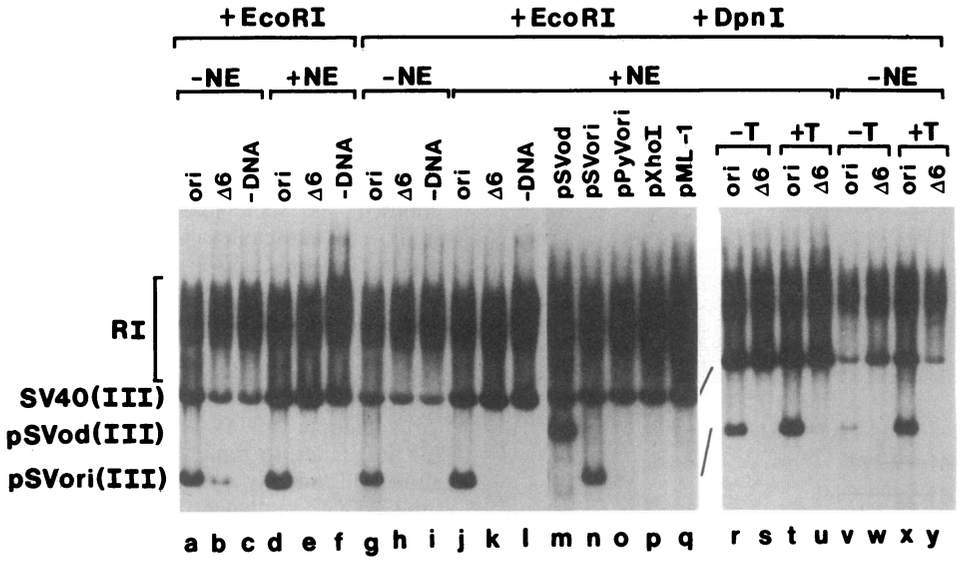

FIG. 2. Requirements for *in vitro* initiation of DNA replication in plasmids containing SV40 DNA. Lanes a–q: A low salt extract of SV40-infected CV-1 cells supplemented with deoxyribo- and ribonucleotides was incubated for 2.5 hr together with a plasmid DNA [pSVori (ori), pSVoriΔ6-1 (Δ6), pSVod, pPyVori, pMLXhoI (pXhoI), or pML-1] in either the absence (−NE) or presence (+NE) of a high salt nuclear extract. The same incubations were also carried out in the absence of plasmid DNA (−DNA). About 13 pmol of dNTPs were incorporated into *Dpn* I-resistant pSVori(III) DNA in the complete system (lane j), and 9 pmol in the absence of nuclear extract (lane g). Lanes r–y: SV40 chromatin was isolated by sedimentation, resuspended in a low-salt extract of uninfected CV-1 cells, supplemented with deoxyribo- and ribonucleotides and with either high-salt nuclear extract from SV40-infected cells or 0.4 μg of purified SV40 T-Ag before incubating with pSVori or pSVoriΔ6-1. DNA was purified from each incubation, digested with either 3 units of *Eco*RI alone, or *Eco*RI plus 1.5 units of *Dpn* I, fractionated by electrophoresis in 0.6% agarose gels (26) and ^{32}P-labeled DNA detected by autoradiography (27). pPyVori contains the polyoma virus *ori* sequence cloned into pML-1 [pdPB503Bg(H); ref. 32]. pMLXhoI (3105 bp) was constructed by insertion of ≈17 tandemly linked *Xho* I linkers into the *Eco*RV site of pML-1. The other plasmids are described in Fig. 1. The positions of form III for SV40, pSVod, and pSVori DNA are indicated, along with replicating intermediate DNA forms cut with *Eco*RI (RI).

tration of pSVod itself (lanes a–c). In contrast, addition of pML-1, pPyVori, or pMLXhoI, none of which replicated in this system, had little or no effect on the replication of pSVod (lanes j–t). Therefore, interference of pSVod replication appeared to result from competition between pSVod and the competitor DNA for binding of one or more initiation factors specifically to the SV40 *ori* region.

The boundaries of this initiation factor binding site were determined by using plasmids containing deletions in and around *ori* (Fig. 1). The plasmids were divided into two

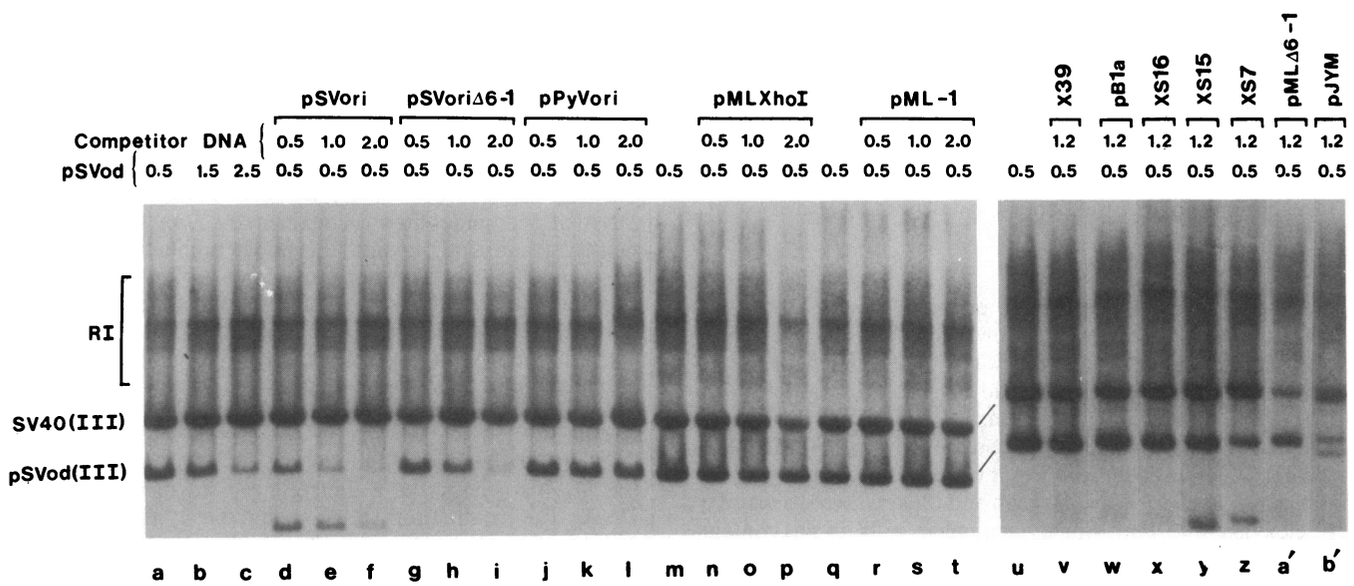

FIG. 3. Inhibition of initiation of SV40 *ori*-dependent DNA replication by specific SV40 sequences. pSVod DNA (0.5 μg per assay) plus competitor DNA (0–2 μg per assay) was incubated for 2.5 hr in the complete *in vitro* DNA replication reaction mixture derived from SV40-infected CV-1 cells. DNA was then purified, digested with *Eco*RI plus *Dpn* I, and analyzed as described in Fig. 2. The amount of pSVod and competitor form I DNA (in μg) added to each reaction mixture is given at the top. Plasmids are described in Fig. 1 or 2. pB1a consists of full-length polyoma virus genome cloned into pML-1 (22). pSVori (lanes d–f), XS15 (lane y), XS7 (lane z), and pJYM (lane b′) replicated under these conditions, thereby producing additional *Dpn* I-resistant ^{32}P-labeled DNA bands when these samples were digested with *Eco*RI. pSVori produced one 2.6-kb fragment, while XS15 and XS7 released a 2.3-kb pBR322 segment and a 5.2-kb SV40 segment that comigrated with endogenous SV40(III) DNA. Digestion of pJYM released two fragments, one slightly smaller than SV40(III) DNA and one smaller than pSVod(III) DNA.

groups according to their size. Plasmids pSVori, pSVoriΔ6-1, pML-1, pPyVori, and pMLXhoI were 2637–3105 bp, which was essentially the same size as pSVod. However, plasmids pJYM (pML-1 containing a full-length SV40 genome), pMLΔ6-1, X39, XS7, XS15, XS16, and pB1a (pML-1 containing a full-length polyoma genome) were 7451–8261 bp. Since these plasmids were about twice the size of pSVod, twice as much competitor DNA had to be added to the reaction to equal the molar concentration of pSVod. Therefore, comparison of the ability of individual plasmids to compete with pSVod for replication factors was confined to members of the same size class. Examples of each competition experiment are shown in Fig. 3. These data, together with additional data not shown, were quantitated, averaged together, and are presented in Fig. 4. The results revealed specific inhibition of SV40 DNA replication at low DNA concentrations and nonspecific inhibition at high DNA concentrations.

pSVoriΔ6-1 containing a 6-bp deletion in the center of *ori*, only partially interfered with pSVod replication in comparison with pSVori, revealing that part of the binding site had been deleted (Fig. 3, compare lane a with lanes d–i; Fig. 4 *Upper*). The same result was obtained when pMLΔ6-1 was compared with pJYM (Fig. 3, compare lane u with lanes a′ and b′; Fig. 4 *Lower*). A larger deletion of *ori*-core, such as the 38-bp deletion carried by plasmid X39, eliminated any significant competition with pSVod replication (Fig. 3, compare lanes u and v; Fig. 4 *Lower*). pB1a was tested as a control for effects of plasmid size on competition. At these DNA concentrations, pB1a, like pPyVori, had no effect on initiation of pSVod replication (Fig. 3, compare lanes u and w; Fig. 4), while the same amount of pJYM completely inhibited replication. These results demonstrated that nucleotides 35–5238, comprising about one-half of *ori*-core, were required for binding one or more factors used to initiate DNA replication. However, progressive deletions that removed the late gene side *ori*-auxiliary sequence (XS7, XS15, XS16) also markedly reduced the ability of competitor DNA to inhibit pSVod replication (Fig. 3, compare lane u with lanes x–z; Fig. 4 *Lower*). These data revealed that the sequence from nucleotide positions 24 to 72 was also involved in binding one or more factors used to initiate SV40 DNA replication. Therefore, the borders of this initiation factor binding site lie somewhere between nucleotides 72 and 108, and nucleotides 5236 and 5243, since deletion of these sequences resulted in only partial interference with DNA replication (Fig. 1).

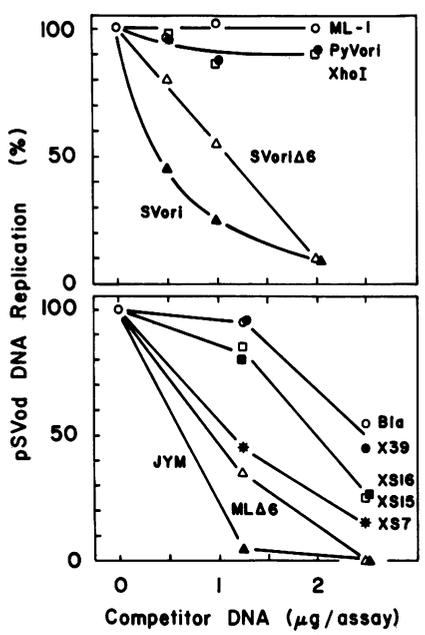

FIG. 4. Quantitative analysis of sequence-specific inhibition of SV40 *ori*-dependent DNA replication. The amount of ^{32}P-labeled DNA in pSVod(III) DNA bands from Fig. 3 and gels not shown was determined by isolating the DNA from the gels and measuring the amount of ^{32}P by scintillation counting. Results from two to four measurements were averaged together and recorded as the fraction of pSVod DNA replication in the presence of various amounts of competitor DNA. Plasmids in the top panel were 2637–3105 bp [pML-1 (○), pPyVori (●), pXhoI (□), pSVoriΔ6-1 (△), pSVori (▲)]. Plasmids in the bottom panel were 7451–8261 bp [pB1a (8261 bp; ○), X39 (●), XS16 (■), XS15 (□), XS7 (*), pMLΔ6-1 (△), pJYM (▲)].

Sufficiently high concentrations of any DNA inhibited SV40 *ori*-dependent DNA replication, presumably through nonproductive binding of proteins required for replication. For example, addition of 1.25 μg of either pB1a or X39 DNA had no effect on pSVod replication, whereas higher concentrations of these plasmids did inhibit replication (Fig. 4 *Lower*). Addition of 5 μg of pB1a DNA inhibited pSVod replication 85%, and the same amount of X39, XS7, XS15, or XS16 inhibited replication by 96–99%.

DISCUSSION

Circular double-stranded DNA molecules containing an intact SV40 *ori*-core sequence (pSVod, pSVori, pJYM, XS15, XS7) initiated replication when provided with SV40 T-Ag and soluble extracts derived from CV-1 cells, a permissive host for SV40 replication. Deletions that removed *ori*-core sequences (pSVoriΔ6-1, pMLΔ6-1, XS16, X39) failed to replicate under these conditions. Moreover, plasmids containing the polyoma virus *ori* (pPyVori, pB1a) instead of the SV40 *ori* also failed to replicate, as did plasmids lacking any eukaryotic origin of replication (pML-1, pXhoI). Thus, initiation of DNA replication under these *in vitro* conditions was specific for the SV40 *ori* sequence. Furthermore, multiple rounds of DNA polymerase α-dependent DNA replication occurred bidirectionally from *ori*. These results are consistent with previous reports on initiation and completion of SV40 DNA replication *in vitro* (21–24) and *in vivo* (1, 2).

DNA substrate competition experiments performed *in vitro* revealed a DNA binding site for a factor(s) present in limiting quantities that is specifically required to initiate SV40 DNA replication. This initiation factor DNA binding site was located between nucleotides 72 and 5243 with borders that may extend as far as nucleotide 108 on the late gene side and nucleotide 5236 on the early gene side (Fig. 1). Since T-Ag is the only viral-specific protein involved in SV40 DNA replication, this result was surprising because it excluded all of T-Ag DNA binding site I and half of site II, and because it included only half of the required *ori*-core sequence but all of the late gene side *ori*-auxiliary sequence. Therefore, the ability of this DNA region to interfere with SV40 DNA replication does not appear to involve binding of T-Ag, as it has been defined traditionally by using purified T-Ag and DNA. DNA that contained most (XS16) or all (XS15) of T-Ag binding sites I and II, as well as DNA that contained all of sites I and III (X39) did not interfere with DNA replication except at high DNA concentrations where interference was no longer sequence specific. Furthermore, other sequences that bind SV40 T-Ag, such as the polyoma *ori* region and tandem *Xho* I linkers (33, 34), failed to interfere with replication under conditions where SV40 *ori* (pSVori) or SV40 *ori* containing a 6-bp deletion (pSVoriΔ6-1) completely inhibited replication (Figs. 3 and 4), revealing that this initiation factor was specific for the SV40 *ori* region.

We suggest that this initiation factor binding site is the target for either a unique subfraction of SV40 T-Ag, or a permissive cell factor required to initiate SV40 (but not polyoma) DNA replication (2). Such a cell factor may form a

specific complex with SV40 T-Ag. Since the late gene side *ori*-auxiliary region can be deleted (7, 9, 10) or displaced up to 56 bp (11) with only a 20–50% reduction in DNA replication *in vivo*, the *ori*-core portion of this binding site is sufficient for replication activity, suggesting that this *ori*-auxiliary sequence promotes binding of the initiation complex to *ori*-core. Elimination of either the *ori*-auxiliary sequence (XS7, XS15) or the *ori*-core portion (X39) of the initiation factor binding site dramatically reduced the ability of plasmids to interfere with replication of other SV40 *ori*-containing plasmids, although removal of the *ori*-core portion of this binding site appeared to have a greater effect (Fig. 4). The SV40 initiation factor binding site, containing one to three 21-bp repeats and a 17-bp A+T-rich sequence, may be analogous to similar binding sites for phage λ and plasmid R6K initiator proteins (35, 36).

Analysis of initiation sites for RNA-primed DNA synthesis in SV40(RI) DNA led to the hypothesis that initiation of DNA synthesis begins at any one of several possible sites within *ori*-core using the same components required to synthesize Okazaki fragments at replication forks (12, 13). Thus, DNA synthesis in *ori* would be initiated by DNA primase–DNA polymerase α (2). This idea is further supported by the observation that initiation of SV40 DNA replication is resistant to α-amanitin and significantly less sensitive to aphidicolin than elongation of SV40(RI) DNA, properties of initiation of DNA synthesis by purified DNA primase–DNA polymerase α (R. S. Decker, M.Y., and M.L.D., unpublished results). However, when presented with single-stranded SV40 DNA fragments containing *ori*, CV-1 cell DNA primase–DNA polymerase α initiated synthesis at sites distinctly different than observed *in vivo* (37). Although site selection and primer synthesis *in vitro* were modulated by the relative ribonucleotide concentrations, initiation sites were primarily determined by DNA sequence (27, 37). Therefore, additional factors that bind to either the template or enzyme are likely required. One or more of these factors together with DNA primase–DNA polymerase α and T-Ag may comprise an initiation complex that binds to the initiation factor binding site, melts the double-stranded DNA into two single-stranded DNA templates, and allows DNA primase–DNA polymerase α to select one of the several possible sites within this region to initiate synthesis. Since transcriptional initiation factor Sp1 binds specifically to the G+C-rich repeats within the initiation factor binding site (38), Sp1 may facilitate activation of SV40 *ori*-core. These G+C-rich repeats are absent from the polyoma virus *ori*.

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