MOLECULAR AND CELLULAR BIOLOGY, Dec. 1983, p. 2203–2210
0270-7306/83/122203-08$02.00/0

Vol. 3, No. 12

Chromatin Structure of Simian Virus 40-pBR322 Recombinant Plasmids in COS-1 Cells

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Received 18 August 1983/Accepted 21 September 1983

To study the nucleoprotein structure formed by recombinant plasmid DNA in mammalian cells, nuclei were isolated from COS-1 cells after transfection with a recombinant (pJI1) containing pBR322 sequences and a segment of simian virus 40 containing information for a nuclease-sensitive chromatin structure. The nuclei were incubated with DNase I. DNA fragments which were the size of linear pJI1 DNA were isolated, redigested with restriction enzymes, fractionated by electrophoresis, and detected by hybridization with nick-translated segments prepared from the plasmid DNA. Two DNase I-sensitive sites were detected in the simian virus 40 portion of the plasmid at the same sites that were DNase I sensitive in simian virus 40 chromatin prepared late after infection of African green monkey kidney (BSC-1) cells. One site extended from the viral origin of replication to approximately nucleotide 40. The 21-base pair repeated sequences were relatively DNase I resistant. A second site occurred over the single copy of the 72-base pair segment present in this plasmid. These results indicate that the nuclease-sensitive chromatin structure does not depend on the presence of viral structural proteins. In addition, late viral proteins added to pJI1-transfected COS-1 cells by superinfection with simian virus 40 caused no change in the distribution of DNase I-sensitive sites in plasmid chromatin. Analysis of transfected plasmid DNA may provide a general method applicable to the study of the chromatin structure of cloned segments of DNA.

The structure of simian virus 40 (SV40) chromatin has been probed by digestion with endonucleases by a number of investigators. A short segment of the viral genome (overlapping the viral promoters and the origin of replication) is hypersensitive to cleavage by endonucleases (2, 4, 5, 9, 22–24, 31, 32, 34, 37). This feature has usually been studied during the late stages of the infection cycle; however, it has also been observed before viral DNA replication (2). It has not been detected in disrupted virus particles (8) or provirions (9).

The nuclease-sensitive chromatin structure is a result of interaction of viral DNA with cellular and, possibly, viral proteins. We have investigated the role of viral structural proteins in the pattern of DNase I cleavage sites by studying the chromatin structure formed by SV40-pBR322 recombinant plasmid DNA transfected into COS-1 cells (7). We show: (i) that recombinant plasmids which have replicated in COS-1 cells can be extracted from the nuclei as nucleoprotein complexes; (ii) that DNase I-sensitive regions exist in the SV40 portion of the plasmid chromatin at sites similar to those observed in viral chromatin late after infection; (iii) that COS-1 cell RNA hybridizes little, if at all, with the late region of the viral genome, suggesting that late proteins cannot be made in these cells and, therefore, are not required for the occurrence of the nuclease-sensitive feature; and (iv) that addition of late proteins, by SV40 superinfection of plasmid-transfected COS-1 cells, does not alter the pattern of DNase I sensitivity in the SV40 sequences of the plasmid, suggesting that late proteins do not modify the chromatin structure of this region.

MATERIALS AND METHODS

Cells and viruses. African green monkey kidney cells (BSC-1) were propagated in minimum essential medium (MEM, Earle salts; MA Bioproducts) supplemented with 10% fetal calf serum (KC Biologicals or Flow Laboratories) in an atmosphere of 5% CO_2 at 37°C. COS-1 cells (7) were a gift from Y. Gluzman and were propagated in the same medium. Wild-type SV40 (strain 776) was used for infection at a multiplicity of 3 to 5 PFU/cell. A mutant of SV40, *in*(Or)-1411 (25) (a gift from T. Shenk), was used at a multiplicity of 0.5 to 1.0 PFU/cell. Virus stocks were grown in BSC-1 cells from isolated plaques.

Enzymes. *Bam*HI was obtained from Bethesda Research Laboratories, and all other restriction enzymes. T4 DNA ligase, and *Escherichia coli* DNA polymerase I were from New England Biolabs. Diges-

tions of DNA were performed under the conditions suggested by the suppliers. RNase A type R was purchased from Worthington Biochemical Corp., and a 2-mg/ml solution was prepared in 0.02 M sodium acetate (pH 5.5)–0.15 M sodium chloride, immersed in a boiling water bath for 15 min, and stored frozen at −20°C. DNase I type D was purchased from Worthington Biochemical Corp., and a 1-mg/ml (2,679 U/ml) solution was prepared in 50% glycerol–0.005 M sodium acetate (pH 5.0) and stored at −20°C. Lysozyme (grade I) was purchased from Sigma Chemical Co. and dissolved in 0.025 M Tris-hydrochloride (pH 7.0) immediately before use.

Construction of pJI1. *in*(Or)-1411 DNA (Fig. 1) was extracted from infected BSC-1 cells by the method of Hirt (10) and purified by cesium chloride-ethidium bromide density equilibrium centrifugation. pBR322 was grown in *E. coli* HB101 under tetracycline selection. Supercoiled circular (form I) plasmid DNA was isolated from chloramphenicol-amplified cultures and purified by using cesium chloride-ethidium bromide centrifugation. A deletion mutant of pBR322 (pBR-dl1329-2517) was constructed to remove sequences poisonous to replication of SV40-pBR322 recombinants in COS-1 cells (13) by digestion with *Pvu*II, followed by *Bal*31 exonuclease, ligation, and transformation of strain HB101. The deleted sequences (determined by the method of Maxam and Gilbert [16]) are indicated in Fig. 1 (nucleotide numbering according to Sutcliffe [28]). A *Pst*I digest of *in*(Or)-1411 DNA was incubated overnight at 16°C in a ligation mixture with *Pst*I-cleaved pBR-dl1329-2517 DNA and T4 DNA ligase. Strain HB101 was transformed with this mixture. Plasmids from tetracycline-resistant, ampicillin-sensitive clones were isolated and analyzed by restriction enzyme digestion. A plasmid which had the structure shown in Fig. 1 was designated pJI1.

Transfection with DNA. Plasmid DNA was introduced into COS-1 cells according to the DEAE-dextran technique of Sompayrac and Danna (26). Briefly, medium from 150-cm^2 dishes of subconfluent COS-1 cells was removed, and cells were washed twice with MEM containing no serum. Next, 6 to 8 ml of MEM containing 200 μg of DEAE-dextran (Sigma Chemical Co.; molecular weight 500,000) and 0.25 to 1 μg of plasmid DNA per ml was added. Incubation was carried out at 37°C for the times indicated in the figure legends. Where indicated, this was followed by incubation at 37°C in medium with 100 μM chloroquine (Sigma Chemical Co.) (14) for the times stated in the figure legends. This medium was removed, the culture was washed with MEM, and the same medium containing 4% fetal calf serum and 40 μg of gentamicin per ml (Schering Corp.) was added.

Isolation of transfected cell nuclei, DNase I digestion, and recovery of plasmid DNA. Nuclei were isolated and digested with DNase I as described by Wu et al. (38) with slight modifications. Transfected cells were scraped from dishes in 5 ml of reticulocyte standard buffer (10 mM Tris-hydrochloride [pH 7.6], 10 mM NaCl, 1.5 mM $MgCl_2$) and collected by centrifugation. The cells were suspended in 2 ml of ice-cold Buffer A (60 mM KCl, 15 mM NaCl, 15 mM Tris-hydrochloride [pH 7.4], 1 mM EDTA, 0.1 mM EGTA [ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*-tetraacetic acid], 0.15 mM spermine, 0.5 mM spermidine, 0.5 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride) per 150-cm^2 dish. The same volume of Buffer A containing 0.5% Nonidet P-40 (Bethesda Research Laboratories) was added. The tube was inverted several times and vortexed briefly to disrupt cells. A 2-ml amount of this nuclear suspension was layered onto 3 ml of Buffer A lacking EDTA and EGTA but containing 1.8 M sucrose and centrifuged at 13,000 rpm in an SW50.1 rotor at 4°C for 15 min. The liquid in the tube

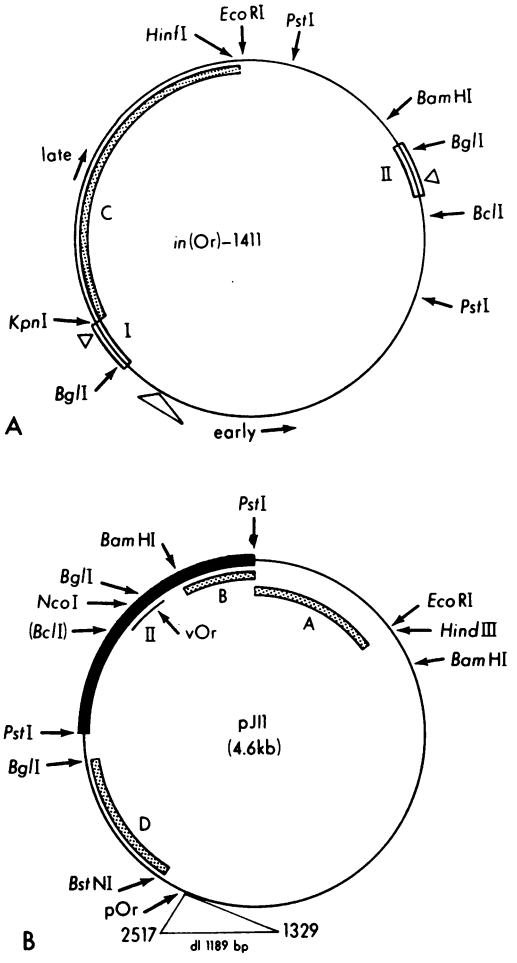

FIG. 1. (A) Physical map of *in*(Or)-1411, a nondefective double-origin mutant of SV40 constructed by T. Shenk (25). I and II are nuclease-sensitive regions in viral chromatin isolated from mutant-infected cells (5). Early and late refer to the transcriptional units of the virus. Wedge-shaped extensions from the circle indicate deleted sequences in *in*(Or)-1411 by comparison with wild-type SV40. The stippled box represents the *Hinf*I-*Kpn*I DNA segment of the virus used to prepare probe C. (B) Physical map of pJI1. A deletion mutant of pBR322 (pBR-dl1329-2517) was prepared as described in the text, and a clone containing the small *Pst*I fragment from *in*(Or)-1411, which spans nuclease-sensitive region II, was designated pJI1. pOr and vOr refer to the pBR322 and SV40 origins of replication, respectively. A, B, and D indicate fragments used to prepare hybridization probes.

was aspirated, and the inside of the tube was wiped dry. The nuclei were suspended in cold DNase I digestion buffer (60 mM KCl, 15 mM NaCl, 15 mM Tris-hydrochloride [pH 7.4], 0.5 mM dithiothreitol, 0.25 mM sucrose, 0.05 mM $CaCl_2$, 3.0 mM $MgCl_2$) at a concentration of 3×10^7 to 4×10^7 nuclei per ml, prewarmed for 1 min at 37°C and digested with DNase I at the concentrations indicated in the figure legends. Digestion was carried out for 3 min at 37°C and then stopped by the addition of sodium dodecyl sulfate and EDTA to final concentrations of 0.5% and 0.01 M, respectively. Low-molecular-weight DNA was isolated by the method of Hirt (10), treated with RNase A (20 μg per ml) for 15 min at 37°C, extracted with phenol saturated with TE (10 mM Tris-hydrochloride [pH 7.4], 1 mM EDTA), extracted with chloroform-octanol (8:1), and precipitated with ethanol. Full-length linear plasmid DNA (form III) was isolated by preparative gel electrophoresis (1.2% agarose) and recovered by hydroxyapatite chromatography after dissolution of the gel slice with 6 M KI in 0.01 M sodium phosphate, pH 7.0 (24). The DNA was dialyzed with several buffer changes against TE and finally against 0.1× SSC (1× SSC is 150 mM NaCl plus 15 mM sodium citrate, pH 7.0), digested with restriction enzymes to localize DNase I cleavage sites, and fractionated by electrophoresis in 1.4% agarose gels.

Blotting, hybridization, and autoradiography. DNA was transferred to nitrocellulose (27) and hybridized in the presence of 50% formamide for 2 to 3 days at 37°C (33) with ^{32}P-labeled probe(s) (prepared by nick translation [21]) indicated in the figure legends. The blots were then washed three times for 5 min each in 2× SSC–0.1% sodium dodecyl sulfate at room temperature and twice for 15 min each in 0.1× SSC–0.1% sodium dodecyl sulfate at 37°C. After drying, each blot was placed directly on Kodak XRP-1 X-ray film with a DuPont Quanta III intensifier and exposed at −70°C for 1 to 3 days.

Purification of RNA and dot blotting. Total cell RNA was isolated from COS-1 cells and SV40-infected BSC-1 cells by lysis in 6 M guanidine isothiocyanate–0.005 M sodium citrate (pH 7.0)–0.1 M β-mercaptoethanol–0.5% Sarkosyl (30), followed by CsCl density centrifugation (6). Portions (3 to 5 μl) of samples containing RNA were dot blotted (after denaturation by immersion in boiling water for 1 min) to nitrocellulose by the procedure of Thomas (29) and hybridized with ^{32}P-labeled probe at 37°C in the presence of 40% formamide, using dextran sulfate to accelerate hybridization (33). After hybridization, the blot was washed, dried, and exposed to X-ray film as described above.

RESULTS

The ability of COS-1 cells to support the replication of plasmid genomes has been utilized to study the expression of cloned genes (18, 20) and the sequence requirements for replication from the SV40 origin (1, 13, 19). Replication of these plasmids and transcription of cloned genes within them are biological tests of the ability of transfected DNA to associate successfully with essential cellular or viral proteins or both. It is reasonable that these plasmids may exist inside the cell in chromatin structures similar to those seen containing SV40 DNA in infected cells.

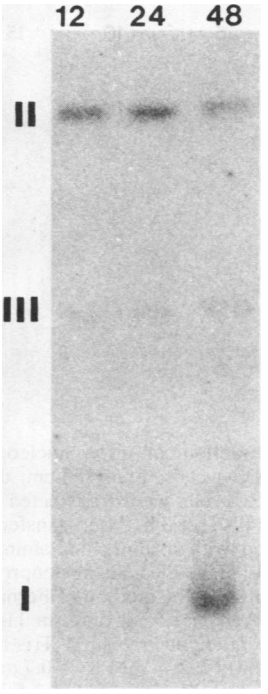

FIG. 2. Replication of pJI1 in COS-1 cells. Subconfluent dishes (20 cm^2) of COS-1 cells were each transfected for 8 h with 10 ng of supercoiled pJI1 DNA in a transfection volume of 2 ml. At the times indicated (hours after the start of transfection) the cells were lysed and the cellular DNA was precipitated by the method of Hirt (10). The supernatant solution was extracted once with phenol and once with chloroform-octanol (8:1) and precipitated with 3 volumes of ethanol. The DNA was suspended, fractionated by electrophoresis through a 1.2% agarose gel, blotted to nitrocellulose paper, and hybridized with a probe made from total SV40 DNA. I, II, and III refer to the supercoiled, relaxed circular, and linear forms of pJI1 DNA, respectively.

Figure 2 shows replication of pJI1 after transfection of COS-1 cells with supercoiled plasmid DNA (13). After 12 h only relaxed circular DNA remained in the cells, in agreement with results reported elsewhere (13, 19). Some replication was detected by 24 h, and a burst was seen between 24 and 48 h. From known amounts of plasmid DNA electrophoresed in the same gel, we estimated that there were 5,000 to 10,000 copies of pJI1 per cell at 48 h (assuming that 5 to 10% of the cells were successfully transfected). pBR322 and pBR-dl1329-2517 did not replicate in COS-1 cells. pJI1 does not code for viral proteins and lacks sequences poisonous to replication in COS-1 cells (13).

Extraction of nucleoprotein containing pJI1 sequences. pJI1-transfected COS-1 cells were

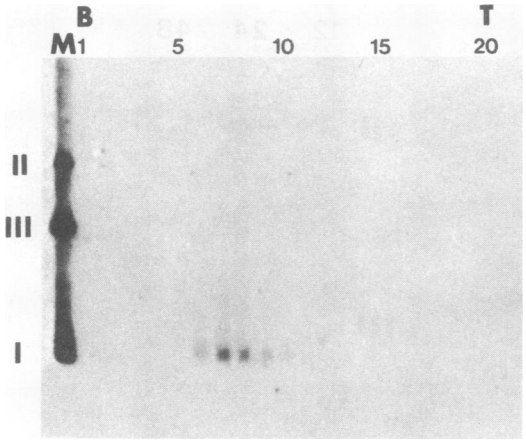

FIG. 3. Extraction of pJI1 nucleoprotein from transfected COS-1 cells. Four 150-cm^2 dishes of subconfluent COS-1 cells were transfected with 2 μg of pJI1 DNA per dish for 3 h. After transfection the cells were incubated with medium containing 100 μM of chloroquine for 2 h. Plasmid nucleoprotein was extracted 40 h later by the method of Fernandez-Munoz et al. (3), layered in 1.5 ml onto an 11-ml 5 to 20% linear sucrose gradient in 25 mM Tris-hydrochloride (pH 7.4)–136 mM NaCl–7 mM KCl–0.7 mM Na_2HPO_4, and centrifuged at 35,000 rpm for 2.5 h (4°C) in an SW41 rotor. Gradient fractions were collected and samples were fractionated by electrophoresis through 1.2% agarose, blotted, and hybridized with probe A (Fig. 1). B and T refer to the bottom and the top of the gradient. Fractions are numbered above the autoradiogram. I, II, and III refer to supercoiled, relaxed circular, and full-length linear pJI1 DNA, respectively.

extracted by the method of Fernandez-Munoz et al. (3), using isotonic conditions. Nucleoprotein molecules containing supercoiled pJI1 DNA were recovered which sedimented in a sucrose gradient to a position similar to that of SV40 chromatin extracted by the same procedure (Fig. 3). pJI1 DNA from peak fractions was digested completely with *Bcl*I, indicating that the DNA had replicated (17).

Distribution of DNase I-sensitive sites in pJI1. In SV40 chromatin a region overlapping the origin of replication and 300 to 400 base pairs (bp) in the late direction from the origin is hypersensitive to DNase I. Since pJI1 occurs as a nucleoprotein structure which replicates in COS-1 cells, it was of interest to determine whether it was also preferentially sensitive to DNase I in the same region of the SV40 sequences. Nuclei from pJI1-transfected COS-1 cells were incubated with DNase I, and low-molecular-weight DNA was recovered. Full-length linear pJI1 DNA was isolated and digested with *Eco*RI or *Bam*HI (Fig. 4). From these patterns it was deduced that DNase I cleaves preferentially in the SV40 sequences of the plasmid at sites near the viral origin of replication. Figure 4, lane D shows the results obtained by DNase I digestion of cesium chloride-ethidium bromide gradient-purified pJI1 DNA, followed by digestion with *Bam*HI. Little, if any, preference of DNase I for the sequences in this region in protein-free DNA was detected. pJI1 chromatin was not cleaved when nuclei were incubated for 30 min at 37°C in DNase I digestion buffer in the absence of added DNase I (data not shown).

Details of cleavage within the SV40 sequences of the plasmid were obtained by using a probe which hybridized with the smaller DNA fragments produced after *Eco*RI cleavage of the linear DNA (Fig. 5A). DNase I cleavage sites were mapped to regions spanning the origin of replication and Goldberg-Hogness sequence and over the single copy of the 72-bp segment. The 21-bp repeated sequences were relatively nuclease resistant (Fig. 6). For comparison, Fig. 5B shows the pattern of DNase I cleavage sites obtained in nuclei from BSC-1 cells 42 h after infection with *in*(Or)-1411 virus. It is clear from these data that DNase I-sensitive regions exist in

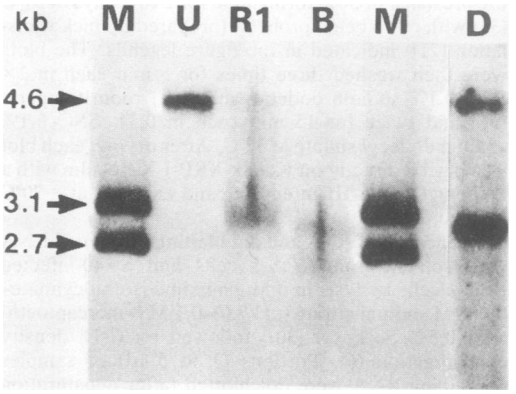

FIG. 4. Distribution of DNase I-sensitive sites in pJI1. Subconfluent COS-1 cells were transfected for 1 h with 5 μg of pJI1 DNA per 150-cm^2 dish and then incubated with medium containing 100 μM of chloroquine for 4 h. After 35 h, nuclei were isolated and digested for 3 min at 37°C with DNase I (25 U/ml). Full-length linear pJI1 DNA (form III) was isolated by preparative agarose gel electrophoresis and samples were digested with *Eco*RI (R1) or *Bam*HI (B), fractionated by agarose gel electrophoresis, blotted, and hybridized with probe D. Lane U shows the isolated form III pJI1 DNA before restriction enzyme digestion. Lane D is 1 μg of 80 to 90% supercoiled pJI1 DNA digested in 0.02 ml with DNase I at 0.05 U/ml for 3 min at 37°C. About 10% of the input DNA was converted to form III. This mixture was then digested with *Bam*HI, fractionated by electrophoresis through an agarose gel, blotted, and hybridized with a probe made from total pJI1 DNA. Lane M is DNA markers prepared from pJI1 DNA.

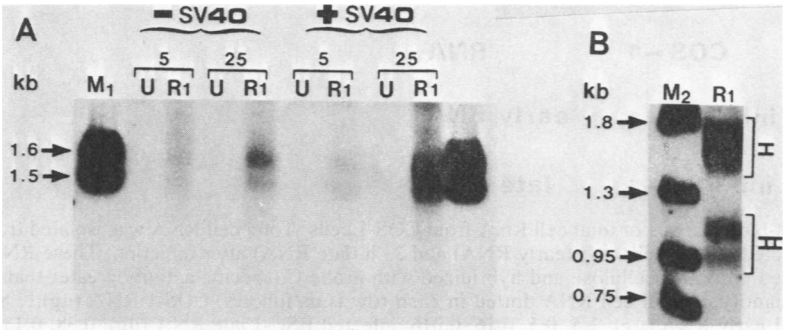

FIG. 5. Comparison of DNase I-sensitive regions in SV40 sequences of pJI1 with those in cells infected with *in*(Or)-1411. (A) Subconfluent dishes of COS-1 cells were each transfected with 5 μg of pJI1 DNA for 2 h. After washing with MEM, medium containing chloroquine was added to one-half of the dishes (−SV40) for an additional 4 h. The remaining dishes (+SV40) were infected with wild-type SV40. Nuclei were isolated 36 h after the start of transfection and digested with DNase I at 5 or 25 U/ml (indicated at the top of the channels) for 3 min at 37°C. Form III pJI1 DNA was recovered, digested with *Eco*RI (R1), fractionated by electrophoresis through a 1.4% agarose gel, blotted, and hybridized with probe A. Only the lower part of the gel is shown. Lane U, isolated form III pJI1 DNA before digestion with *Eco*RI. Lane M1, DNA markers *Eco*RI to *Nco*I fragment from pJI1 (1.5 kilobases) and *Bam*HI to *Bam*HI fragment from pJI1 (1.6 kilobases). (B) Nuclei from *in*(Or)-1411-infected BSC-1 cells were isolated 42 h after infection and digested with DNase I at 25 U/ml for 3 min at 37°C. Form III viral DNA was purified and digested with *Eco*RI. After electrophoresis, the DNA was transferred to nitrocellulose and hybridized simultaneously with probe B and a probe similar to C but extending from *Kpn*I to *Eco*RI. Lane M2. DNA markers *Eco*RI to *Bgl*I, *Eco*RI to *Bcl*I, *Eco*RI to *Bgl*I, and *Eco*RI to *Bam*HI fragments from *in*(Or)-1411; 1.8, 1.3, 0.95, and 0.75 kilobases, respectively. I and II refer to clusters of DNase I cutting sites in *in*(Or)-1411 chromatin (see Fig. 1).

pJI1 in nuclei from COS-1 cells with a pattern indistinguishable from that of *in*(Or)-1411 chromatin late after infection.

The role of viral late proteins in the nuclease-sensitive region. The organization of SV40 sequences in COS-1 cells suggests that late genes should not be expressed to produce viral structural proteins (7). We probed for transcripts containing viral late sequences by isolating total RNA from COS-1 cells, dot blotting onto nitrocellulose, and hybridizing with a late region-specific probe which had been labeled by nick translation (Fig. 7). By comparing dot intensities we concluded that transcripts in COS-1 cells contain less than 0.2% of the level of late viral sequences found in SV40-infected BSC-1 cells late after infection.

Further experiments were performed to evaluate the effect of adding SV40 late proteins to plasmid-transfected COS-1 cells (Fig. 5A). The pattern of cleavage of pJI1 by DNase I was the same whether or not the transfected cells were superinfected with SV40.

DISCUSSION

It is evident that plasmids in transfected COS-1 cells have features in common with SV40 chromatin in infected cells. First, plasmids with a SV40 origin are capable of autonomous replication (1, 13, 19). Second, transcription of genes contained in these plasmids is initiated at the correct start sites and transcriptional regulation of these genes is maintained (18, 20). Third, nucleoprotein containing supercoiled plasmid DNA can be extracted from COS-1 cells, and those nucleoprotein molecules have sedimentation properties similar to SV40 chromatin. Fourth, DNase I cleaves plasmid DNA in COS-1

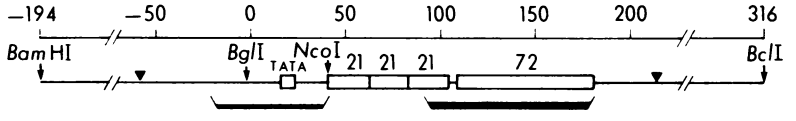

FIG. 6. Regions of DNase I cleavage in SV40 sequences of pJI1 and in region II of *in*(Or)-1411 late after infection. Brackets indicate the regions which are preferentially cleaved by DNase I in both pJI1 and *in*(Or)-1411 chromatin. The thickness of the lines indicates approximate relative frequency of cleavage. Numbering is in base pairs from the center of the 27-bp palindrome, with positive values in the late direction with respect to the inserted origin region. The 21-bp repeated sequences and the 72-bp segment which is repeated in wild-type SV40 but not in either segment of *in*(Or)-1411 or pJI1 are shown by numbers and boxes. TATA refers to the Goldberg-Hogness sequences. ▼, Joints of the inserted origin region (25).

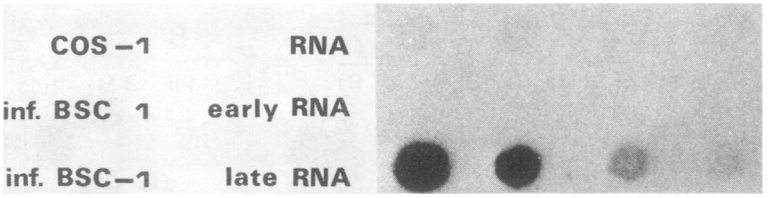

FIG. 7. Dot-blot analysis of total cell RNA from COS-1 cells. Total cell RNA was isolated from COS-1 cells and SV40-infected BSC-1 cells at 8 (early RNA) and 33 h (late RNA) after infection. These RNA preparations were dot blotted onto nitrocellulose and hybridized with probe C (specific activity greater than 10^9 cpm/μg of DNA). The quantity of total cell RNA dotted in each row is as follows. COS-1 RNA (μg): 7.8, 4.7, 1.6, 0.5; infected BSC-1 early RNA (μg): 1.5, 0.5, 0.16, 0.016; infected BSC-1 late RNA (μg): 0.38, 0.13, 0.04, 0.013.

nuclei preferentially in the SV40 sequences at sites similar to those cleaved in SV40 chromatin late after infection (2, 22, 23).

Our experiments demonstrate that the nuclease-sensitive chromatin structure of SV40 forms in the absence of viral late proteins and that the DNA does not need to be delivered as nucleoprotein packaged in a virus particle to acquire a nuclease-sensitive feature. These results are in agreement with those of Saragosti et al. (22), who showed that the DNase I cleavage pattern of *tsB*201 chromatin did not change when infected cells were shifted to the nonpermissive temperature late after infection, indicating that maintenance of the pattern of DNase I-sensitive sites does not depend on functional major capsid protein (VP1). We have extended their observations by showing that none of the viral structural proteins are required for the establishment of the nuclease-sensitive region. Llopis and Stark (12) reported evidence that VP2 or VP3 or both confer altered transcriptional properties on SV40 nucleoproteins late after infection. We did not detect a change in the pattern of DNase I-sensitive sites in pJI1 when late proteins were provided by SV40 superinfection. Since transcriptional complexes comprise a small percentage of the SV40 nucleoprotein molecules late after infection (0.5 to 1% [11]), it is likely that we would not have detected altered structural features if they were limited to this subfraction. It is also possible that late proteins can influence transcriptional activity by binding to SV40 sequences not included in pJI1 or that binding of late protein(s) in this region of the genome may not change the pattern of DNase I cleavage sites.

Our results with plasmids do not allow us to evaluate the role which the process of DNA replication might have in the occurrence of the nuclease-sensitive sites. The DNase I cleavage pattern seen in Fig. 5A was also observed at earlier times after transfection (seen clearly at 24 h and, faintly, at 10 h after transfection); however, its appearance at early times was partially obscured by a background of degradation products which were apparently random. The emergence of DNase I specificity at later times after transfection can be explained by the preferential replication of undegraded DNA molecules and does not imply that replication must occur before the nuclease-sensitive sites can form.

In agreement with Crémisi (2), we found that the nuclease-sensitive region is established in the absence of replication when the viral DNA is introduced in virions (data not shown). Both origin regions of *in*(Or)-1411 were DNase I sensitive in nuclei isolated from BSC-1 cells which had been incubated from the time of infection and during the isolation of nuclei (isolated 7 h after the start of the infection) with 25 μg of cytosine arabinoside per ml (15). On the other hand, the presence of the nuclease-sensitive region may enhance the function of the replication origin. Mutations which displace the nuclease-sensitive region away from the origin reduce the rate of replication (manuscript in preparation).

No role for large T antigen in the nuclease-sensitive chromatin structure has been defined. When cells were infected with SV40 mutant *tsA*58 and shifted to the nonpermissive temperature late after infection, the pattern of DNase I cleavage sites did not change (23; J. P. Hartmann and W. A. Scott, unpublished data). In addition, deletion mutants which lack T antigen binding site 2 retain the nuclease-sensitive feature (5).

The sequences required to form nuclease-sensitive sites are contained in a limited segment of viral DNA. We have previously shown (5, 37) that sequences between map positions 0.65 and 0.72 on the SV40 genome contain *cis*-acting information sufficient to organize the nuclease-sensitive chromatin structure, and Fromm and Berg (4) have shown that a segment of the SV40 genome containing the 72-bp repeated sequences organized a DNase I-sensitive site when placed at different positions in the viral genome. In this paper, we have shown that the sequences can also generate the nuclease-sensitive structure when introduced into cells as part

of a plasmid DNA molecule. These results are in agreement with the view that chromatin structure is determined, in large part, by the primary sequence of a segment of DNA and the consequent secondary structure (35, 36).

The method of experimentation used here should be appropriate for studying the chromatin structure of other cloned segments of DNA. The effects of mutations (e.g., deletions, insertions, and point mutations) on nuclease sensitivity of a variety of cellular or viral DNA segments can be conveniently determined by analysis in plasmids.

ACKNOWLEDGMENTS

We thank T. Shenk for providing a seed stock of *in*(Or)-1411 and Y. Gluzman for providing COS-1 cells. We thank C. Walter for valuable technical assistance, K. Ness for teaching us the blotting procedure, R. Gerard for teaching the DNA sequencing procedure, and B. Montelone for helpful comments on the manuscript.

This research was supported by Public Health Service grant AI-12852 from the National Institutes of Health. W.A.S. is the recipient of Research Career Development award AM-00549 from the National Institutes of Health.

ADDENDUM IN PROOF

We have recently learned (personal communication) that S. Cereghini and M. Yaniv have also introduced an SV40 hybrid lacking viral late genes into COS-1 cells and observed a pattern of DNase I-hypersensitive sites similar to that observed in lytic infection.

LITERATURE CITED

1. **Bergsma, D. J., D. M. Olive, S. W. Hartzell, and K. N. Subramanian.** 1982. Territorial limits and functional anatomy of the simian virus 40 replication origin. Proc. Natl. Acad. Sci. U.S.A. **79**:381–385.
2. **Crémisi, C.** 1981. The appearance of DNase I-hypersensitive sites at the 5' end of the late SV40 genes is correlated with the transcriptional switch. Nucleic Acids Res. **9**:5949–5964.
3. **Fernandez-Munoz, R., M. Coca-Prados, and M.-T. Hsu.** 1979. Intracellular forms of simian virus 40 nucleoprotein complexes. I. Methods of isolation and characterization in CV-1 cells. J. Virol. **29**:612–623.
4. **Fromm, M., and P. Berg.** 1983. Simian virus 40 early- and late-region promoter functions are enhanced by the 72-base-pair repeat inserted at distant locations and inverted orientations. Mol. Cell. Biol. **3**:991–999.
5. **Gerard, R. D., M. Woodworth-Gutai, and W. A. Scott.** 1982. Deletion mutants which affect the nuclease-sensitive site in simian virus 40 chromatin. Mol. Cell. Biol. **2**:782–788.
6. **Glisin, V., R. Crkvenjakov, and C. Byus.** 1974. Ribonucleic acid isolated by cesium chloride centrifugation. Biochemistry **13**:2633–2637.
7. **Gluzman, Y.** 1981. SV40-transformed simian cells support the replication of early SV40 mutants. Cell **23**:175–182.
8. **Hartmann, J. P., and W. A. Scott.** 1981. Distribution of DNase I-sensitive sites in simian virus 40 nucleoprotein complexes from disrupted virus particles. J. Virol. **37**:908–915.
9. **Hartmann, J. P., and W. A. Scott.** 1983. Nuclease-sensitive sites in the two major intracellular simian virus 40 nucleoproteins. J. Virol. **46**: 1034–1038.
10. **Hirt, B.** 1967. Selective extraction of polyoma DNA from infected mouse cell cultures. J. Mol. Biol. **26**:365–369.
11. **Llopis, R., F. Perrin, F. Bellard, and P. Gariglio.** 1981. Quantitation of transcribing native simian virus 40 minichromosomes extracted from CV1 cells late in infection. J. Virol. **38**:82–90.
12. **Llopis, R., and G. R. Stark.** 1981. Two deletions within genes for simian virus 40 structural proteins VP2 and VP3 lead to formation of abnormal transcriptional complexes. J. Virol. **38**:91–103.
13. **Lusky, M., and M. Botchan.** 1981. Inhibition of SV40 replication in simian cells by specific pBR322 DNA sequences. Nature (London) **293**:79–81.
14. **Luthman, H., and G. Magnusson.** 1983. High efficiency polyoma DNA transfection of chloroquine treated cells. Nucleic Acids Res. **11**:1295–1308.
15. **Manteuil, S., H. Kopecka, J. Caraux, A. Prunell, and M. Girard.** 1974. *In vivo* incorporation of cytosine arabinoside into simian virus 40 DNA. J. Mol. Biol. **90**:751–756.
16. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol. **65**:499–560.
17. **McClelland, M.** 1983. The effect of site specific methylation on restriction endonuclease cleavage (update). Nucleic Acids Res. **11**:r169–r173.
18. **Mellon, P., V. Parker, Y. Gluzman, and T. Maniatis.** 1981. Identification of DNA sequences required for transcription of the human α1-globin gene in a new SV40 host-vector system. Cell **27**:279–288.
19. **Myers, R. M., and R. Tjian.** 1980. Construction and analysis of simian virus 40 origins defective in tumor antigen binding and DNA replication. Proc. Natl. Acad. Sci. U.S.A. **77**:6491–6495.
20. **Pelham, H. R. B.** 1982. A regulatory upstream promoter element in the Drosophila hsp 70 heat-shock gene. Cell **30**:517–528.
21. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
22. **Saragosti, S., S. Cereghini, and M. Yaniv.** 1982. Fine structure of the regulatory region of simian virus 40 minichromosomes revealed by DNase I digestion. J. Mol. Biol. **160**:133–146.
23. **Saragosti, S., G. Moyne, and M. Yaniv.** 1980. Absence of nucleosomes in a fraction of SV40 chromatin between the origin of replication and the region coding for the late leader RNA. Cell **20**:65–73.
24. **Scott, W. A., and D. J. Wigmore.** 1978. Sites in simian virus 40 chromatin which are preferentially cleaved by endonucleases. Cell **15**:1511–1518.
25. **Shenk, T.** 1978. Construction of a viable SV40 variant containing two functional origins of DNA replication. Cell **13**:791–798.
26. **Sompayrac, L. M., and K. J. Danna.** 1981. Efficient infection of monkey cells with DNA of simian virus 40. Proc. Natl. Acad. Sci. U.S.A. **78**:7575–7578.
27. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
28. **Sutcliffe, J. G.** 1979. Complete nucleotide sequence of the *Escherichia coli* plasmid pBR322. Cold Spring Harbor Symp. Quant. Biol. **43**:77–90.
29. **Thomas, P. S.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. U.S.A. **77**:5201–5205.
30. **Ullrich, A., J. Shine, J. Chirgwin, R. Pictet, E. Tischer, W. J. Rutter, and H. M. Goodman.** 1977. Rat insulin genes: construction of plasmids containing the coding sequences. Science **196**:1313–1319.
31. **Varshavsky, A. J., O. Sundin, and M. Bohn.** 1979. A stretch of "late" SV40 viral DNA about 400 bp long which includes the origin of replication is specifically exposed in SV40 minichromosomes. Cell **16**:453–466.
32. **Varshavsky, A. J., O. H. Sundin, and M. J. Bohn.** 1978. SV40 viral minichromosome: preferential exposure of the origin of replication as probed by restriction endonucle-

ases. Nucleic Acids Res. 5:3469–3477.
33. **Wahl, G. M., M. Stern, and G. R. Stark.** 1979. Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl-paper and rapid hybridization by using dextran sulfate. Proc. Natl. Acad. Sci. U.S.A. 76:3683–3687.
34. **Waldeck, W., B. Föhring, K. Chowdhury, P. Gruss, and G. Sauer.** 1978. Origin of DNA replication in papovavirus chromatin is recognized by endogenous endonuclease. Proc. Natl. Acad. Sci. U.S.A. 75:5964–5968.
35. **Wasylyk, B., P. Oudet, and P. Chambon.** 1979. Preferential *in vitro* assembly of nucleosome cores on some AT-rich regions of SV40 DNA. Nucleic Acids Res. 7:705–713.
36. **Weintraub, H.** 1983. A dominant role for DNA secondary structure in forming hypersensitive structures in chromatin. Cell 32:1191–1203.
37. **Wigmore, D. J., R. W. Eaton, and W. A. Scott.** 1980. Endonuclease-sensitive regions in SV40 chromatin from cells infected with duplicated mutants. Virology 104:462–473.
38. **Wu, C., P. M. Bingham, K. J. Livak, R. Holmgren, and S. C. R. Elgin.** 1979. The chromatin structure of specific genes. I. Evidence for higher order domains of defined DNA sequence. Cell 16:797–806.