A Transcription Factor from Simian Virus 40 Chromosomes Which Activates the Viral Late Promoter In Vitro

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We have characterized a transcription factor, obtained from simian virus 40 (SV40) chromosomes, which activates transcription from the SV40 late promoter in vitro. The late promoter-activating factor was distinct from SV40 T antigen as judged by its behavior on chromatography on hydroxylapatite; it was not recognized by anti-T antibodies, while T antigen itself was recognized. T antigen from SV40 chromosomes, on the other hand, abolished transcription in vitro from the early promoter. In DNase I footprinting experiments, a partially purified late promoter-activating factor preparation protected a region of DNA centered on SV40 nucleotide 270, which is between the repeated 72-base-pair enhancer and the major late RNA start site. Proteins from HeLa cells did not give the same footprint at this position. Gel mobility shift assays showed that proteins from SV40-infected CV-1 cells form a complex with DNA containing this binding site. The complex has a different rate of gel migration and a higher stability than complexes formed with proteins from uninfected cells.

The simian virus 40 (SV40) genome comprises two transcription units. One contains the early genes, and the other, oriented in the opposite direction, contains the late genes. During lytic infection, transcription of SV40 is regulated: before viral DNA replication starts, transcription is mostly of the early genes. After the viral DNA begins to replicate, the late promoter is activated and transcription is predominantly of the late genes (15, 43). The SV40 early-gene product, large T antigen, is responsible for repression of early transcription and, indirectly or directly, for the increase in late transcription (8, 23, 26).

To activate transcription from the late viral promoter, SV40 does not use just one molecular mechanism (2, 27, 28, 38, 41). Rather, multiple DNA sequences between the origin of DNA replication around nucleotide position 1 and the predominant late RNA start site at position 325 have been implicated. These sequences include the origin of replication (13, 27), the 21-base-pair guanosine-cytosine-rich repeats (10, 18, 21, 22, 28, 36, 37, 44), parts of the 72-base-pair repeated enhancer (17, 18, 23, 27), and the region from the enhancer up to and beyond the major late RNA initiation site (5, 11, 17, 27). This initiation site does not have a typical TATA box, and RNA also initiates, albeit less frequently, at other sites between this site and the DNA origin (11, 20).

Most of the DNA sequences involved in control of late transcription probably function by interacting with regulatory proteins. We reasoned that such proteins might be associated with transcribed SV40 chromosomes in the late phase of the lytic cycle. We have shown earlier (6, 41) that the activity of the late promoter in SV40 chromosomes is determined by a combination of a stable nucleoprotein structure and *trans*-acting factors. In the work presented here, we have characterized a transcriptional regulatory factor (the late promoter-activating factor, LAF), prepared from SV40 chromosomes, which is apparently distinct from T antigen and which activates transcription from the major late SV40 RNA start site in vitro.

MATERIALS AND METHODS

SV40 chromosomes. SV40 chromosomes were extracted from infected CV-1 cells 36 h after infection as described previously (6, 41), except that cells were washed with KP buffer containing 10 mM K_2HPO_4 - KH_2PO_4 (pH 7)-1 mM dithiothreitol, in place of HBE buffer. The viral chromosomes were extracted into KP buffer containing in addition 10% (vol/vol) glycerol. Hydroxylapatite (Bio-Rad HTP) was washed in KP buffer, and equal volumes of the settled slurry and nuclear extract containing SV40 chromosomes were mixed at 4°C for 15 min. The hydroxylapatite was recovered by brief centrifugation, and proteins were eluted by suspending it in buffer containing 0.1 M K_2HPO_4 - KH_2PO_4 (pH 7) plus either 0.4 M KCl alone or 1 M KCl plus 5 M urea. The suspended hydroxylapatite was mixed with the eluting buffer for 15 min at 4°C and then repelleted by brief centrifugation. The supernatants were dialyzed overnight at 4°C against buffer D (16) containing 20 mM HEPES (*N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid)-KOH (pH 7.9), 20% (vol/vol) glycerol, 0.1 M KCl, 0.2 mM EDTA, and 0.5 mM dithiothreitol. Extracts were generally stored on ice and used soon after preparation. Sometimes extracts lacked the transcriptional repression activity of T antigen or late promoter-activating activity for unknown reasons. Protein concentrations were determined with the Bio-Rad protein assay with bovine serum albumin as standard.

Nuclear extracts and transcription in vitro. Extracts of HeLa cells were prepared as described by Dignam et al. (16). Extracts of CV-1 cells were prepared by the same method, modified slightly for use with cells grown as monolayers. Transcription reactions, generally in a volume of 50 μ l, analysis of RNA products by denaturation with glyoxal, and electrophoresis through 2% agarose gels were as described previously (16, 41).

Proteins. Proteins were analyzed by electrophoresis through 10% (wt/vol) polyacrylamide-sodium dodecyl sulfate gels (29). After electrophoresis the proteins were transferred electrophoretically to a sheet of nitrocellulose by using a Bio-Rad Trans Blot cell run overnight at 30 V at 4°C. Molecular weight markers were stained with 3% Ponceau red in 5% trichloroacetic acid and then washed with water.

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The filter was then placed in phosphate-buffered saline plus 5% (wt/vol) dried milk (Blotto [24]) with three changes of 10 min each. Ascites fluids containing monoclonal anti-T antibodies 402, 405, 416, 423, and 430 were prepared as described previously (41). The hybridoma cell lines were very kindly provided by E. Harlow.

A mixture of equal volumes of each ascites fluid was diluted 1/250 in Blotto, and the nitrocellulose filter was incubated in this solution for 3 h at room temperature on a shaker. The filter was washed three times for 10 min each in 20 mM Tris hydrochloride (pH 8.5)–100 mM NaCl–5% dried milk and then incubated with ^{125}I -labeled protein A (a generous gift of J. Bouvier), 2×10^5 cpm/ml, 0.1 ml/cm² of filter, in a sealed bag, in the same buffer for 2 h. The filter was washed twice for 3 min each and then twice for 10 min each in the same buffer but without the dried milk, dried, and exposed for autoradiography with Kodak XAR film and an intensifying screen.

To remove T antigen from cellular extracts, fixed *Staphylococcus aureus* (The Enzyme Center) was first suspended in phosphate-buffered saline at 10% (wt/vol) and washed three times by brief centrifugation in the Eppendorf microcentrifuge and suspension in the same buffer. The final pellet was suspended at 20% (wt/vol) in hamster anti-T serum (a kind gift of H. Türler) and kept with mixing at 4°C. The *Staphylococcus aureus* with bound antibody was washed four times with buffer D (see above) by pelleting and suspending. The final pellet was suspended in the solution from which T antigen was to be removed. After incubation overnight on ice, the suspension was centrifuged to pellet the *Staphylococcus aureus*, and the supernatants were tested for content of T antigen by immunoblotting (see above) or for effects on transcription.

DNase I footprinting. The *Hind*III-*Hpa*II origin-containing fragment of SV40 DNA was cloned between the *Hind*III and *Acc*I sites of the vector pSP65. The plasmid was cut at the *Bam*HI site (which is adjacent to the former *Hpa*II site in the insert), labeled by using the Klenow fragment of DNA polymerase (33) (the labeled nucleoside triphosphate being [α - ^{32}P]dATP) digested with *Hind*III, and the shorter DNA fragment was isolated by preparative agarose gel electrophoresis. Protein-DNA binding reactions (20 μ l) contained 1 to 3 ng of labeled DNA fragment, protein extract and buffer D to a combined volume of 12 μ l, and 0.5 mM dithiothreitol. Reactions containing unfractionated nuclear extracts had in addition 1 μ g of poly(dI-dC). Some binding reactions were done in the presence of 1 mM MgCl_2 . This did not alter the footprints obtained but did increase the activity of endogenous nucleases in the extracts. After 15 min at room temperature, MgCl_2 was added to a final concentration of 6 mM, followed immediately by addition of DNase I (from Boehringer Mannheim Biochemicals; the amount must be determined empirically, but in general it was 0.1 to 0.3 U). The incubation was continued for 30 s. The digestion was stopped by the addition of 100 μ l of a solution containing 100 mM Tris hydrochloride (pH 8), 1% (wt/vol) sodium dodecyl sulfate, 10 mM EDTA, 100 mM NaCl, 25 μ g of tRNA per ml, and 200 μ g of pronase per ml. After 15 min at 37°C, the reactions were extracted with phenol-chloroform (1:1) and adjusted to 0.3 M in sodium acetate, and the nucleic acids were precipitated at -70°C for 20 min with 2.5 volumes of ethanol. After centrifugation for 10 min in the Eppendorf microfuge at 4°C, the pellets were dried, dissolved in gel loading buffer containing 80% (vol/vol) formamide–1 $\times$ Tris-borate-EDTA buffer (33)–0.1% bromophenol blue, heated for 3 min in boiling water, and then analyzed by electropho-

resis through a 6% polyacrylamide sequencing gel as described by Sanger and Coulson (40).

Gel mobility shift assay. The SV40 DNA fragment *Bst*NI-*Kpn*I (nucleotides 234 to 294) was cloned in the *Sma*I site of pSP65. The fragment was excised with *Eco*RI and *Bam*HI and labeled with ^{32}P by using Klenow polymerase as described above. Standard binding reactions (20 μ l) contained protein extract (dialyzed against buffer D of Dignam et al. [16]) plus buffer D to a combined volume of 12 μ l, 100 μ g of bovine serum albumin per ml, and 1 ng of ^{32}P -labeled DNA probe. After 30 min of incubation at room temperature, the mixture was loaded on a 2% agarose gel in 0.25 $\times$ Tris-borate-EDTA buffer (33) and electrophoresed at 5 V/cm, and the gel was dried and autoradiographed.

RESULTS

Extraction of proteins from SV40 chromosomes. Nuclei were prepared from CV-1 cells 36 h after infection by SV40, and the viral chromosomes were isolated by diffusion from the nuclei at low ionic strength. SV40 chromosomes were bound to hydroxylapatite, and the proteins were eluted by washing with buffer containing increasing concentrations of KCl or urea. Eluted proteins were dialyzed against buffer D (see Materials and Methods); they were free of detectable contamination by RNA or DNA as judged by agarose gel electrophoresis and staining with ethidium bromide.

Effect of SV40 chromosomal proteins on the activity of viral promoters in vitro. When SV40 DNA is transcribed in vitro by nuclear extracts of HeLa cells, the early promoter is up to 10-fold stronger than the late promoter. With isolated SV40 chromosomal templates, on the other hand, the late promoter is 3- to 10-fold more active than the early promoter (6, 41). To test the effect of added SV40 chromosomal proteins on the pattern of transcription of purified SV40 DNA, *Pst*I-cut SV40 DNA was incubated with HeLa nuclear extract in the absence or presence of different protein fractions. Figure 1 shows the resulting transcripts. We have identified (41) the RNA of about 2 kilobases (kb) as a runoff transcript from the early promoter to the *Pst*I site at position 3204 and the RNAs of about 1.7 and 1.8 kb as runoff transcripts from the major late start site and from a minor late start site at position 170, respectively, to the *Pst*I site at position 1988. Transcription of the DNA alone gave principally the 2-kb early RNA. With addition of the 0.4 M KCl eluate from hydroxylapatite, the 2-kb RNA was still the main product, although in this experiment there was an overall inhibition of transcription. Addition of the 1 M KCl–5 M urea eluate, in contrast, resulted in a complete switch in the transcription pattern. Transcription from the early promoter was abolished, while that from the major late start site was stimulated by a factor of about 10. Transcription from the minor late site was also stimulated. (Above the early and late transcripts two other bands were seen. The uppermost, of 4 kb, corresponded in size to the whole length of the DNA template fragment. The lower was of about 3 kb. They have not been studied further.)

The magnitude of the effect on transcription of adding the 1 M KCl–5 M urea eluate depended on the amount of extract (Fig. 2), with an optimum of 10 μ l of extract in a 50- μ l reaction.

T antigen and the transcriptional switch. SV40 chromosomes bound to hydroxylapatite were washed with buffer containing 0.4 M KCl, 1 M KCl, or 1 M KCl–5 M urea. For each fraction, the effects on transcription in vitro and the presence or absence of T antigen were tested (Fig. 3).

FIG. 1. Effect of proteins extracted from SV40 chromosomes on transcription from *Pst*I-cut SV40 RNA. The positions in the agarose gel of the 2.04-kb early transcript and the 1.66- and 1.82-kb late transcripts (38) are shown at the top left. Transcription reactions were supplemented as follows: buffer alone (lane 1); the 0.4 M KCl eluate of hydroxylapatite-bound SV40 chromosomes (10 μ g) (lane 2), and the 1 M KCl-5 M urea eluate of these chromosomes (7.5 μ g) (lane 3). These extracts were added to the transcription mixtures before the HeLa extract. Scheme showing the transcription pattern of the larger *Pst*I fragment of SV40 DNA is at the bottom left. The early transcript (synthesized leftwards) and the two late transcripts (synthesized rightwards) are made by runoff transcription from the SV40 origin-promoter region O to the two *Pst*I sites. Densitometric scans of the sections of lanes 1 and 3 containing early and late transcripts are on the right.

Transcription in the presence of the 0.4 M KCl fraction again gave predominantly the early RNA. In the presence of the 1 M KCl fraction a fourfold stimulation of late transcription was seen, while early transcription remained at the same level. On addition of the 1 M KCl-5 M urea fraction, early transcription was stopped, while the late transcription remained.

A sample of each protein fraction was electrophoresed in a sodium dodecyl sulfate-polyacrylamide gel and transferred to a sheet of nitrocellulose. The sheet was probed with a mixture of monoclonal antibodies against T antigen followed by 125 I-labeled protein A to localize bound antibody. T antigen was found only in the 1 M KCl-5 M urea fraction (Fig. 3, lane c).

A 1 M KCl-5 M urea eluate of hydroxylapatite-bound SV40 chromosomes was depleted of T antigen by incubating it with anti-T immunoglobulin immobilized on fixed *Staphylococcus aureus*. Polyacrylamide gel electrophoresis, transfer, and probing with anti-T antibodies as described above showed that the depletion was effective (Fig. 4, lane c). When the T-antigen-depleted eluate was tested for its effect on transcription in vitro, it no longer abolished early transcription, yet it still stimulated the late transcription (Fig. 4, lane 3).

These results show that the factor activating the late promoter did not behave like the SV40 T antigen with respect to elution from hydroxylapatite or to reaction with anti-T antiserum. Inhibition of the early promoter, on the other hand, could be fully accounted for by the presence of T antigen.

Binding of SV40 chromosomal proteins to the viral origin-

FIG. 2. Effect of varying the concentration of SV40 chromosomal proteins on transcriptional specificity. *Pst*I-cut SV40 DNA was transcribed, and the products were analyzed by agarose gel electrophoresis and autoradiography as for Fig. 1. Standard transcription reactions were supplemented with 0, 5, 10, or 15 μ l of a 1 M KCl-5 M urea eluate of hydroxylapatite-bound SV40 chromosomes with 0.75 μ g of protein per μ l. The reactions contained a constant 70 mM KCl except for the reaction in lane 4, in which the volume of added eluate brought the KCl concentration to 80 mM. Some of the inhibition with 15 μ l of eluate was probably due to the increased KCl concentration. Changes in KCl concentration between 50 and 70 mM had no effect on the ratio of early to late transcripts (data not shown). E and L, Positions of early and late transcripts, respectively.

promoter region. We used DNase I footprinting to look at how the proteins which eluted from hydroxylapatite-bound SV40 chromosomes interact with the region of viral DNA between the DNA replication origin and the major late RNA start site. Several proteins from HeLa cells which bind to this region are already known, including Sp1 (12) and LSF (28), which bind to the guanosine-cytosine-rich 21-base-pair repeats, and AP-1, AP-2, AP-3, AP-4 (34, 35), EBP20 (25), octamer-binding proteins (7, 39 and references therein), and the GT-II proteins (45), which bind to different sites within the SV40 enhancer. Using an *Hpa*II-*Hind*III fragment of SV40 DNA labeled adjacent to the *Hpa*II site, we first looked at DNA binding of factors in the nuclear extract of

FIG. 3. T antigen and transcriptional regulation by proteins from SV40 chromosomes. Left: transcription reactions, as shown in Fig. 1, supplemented with proteins eluted from hydroxylapatite-bound SV40 chromosomes by using 0.4 M KCl (lane 1), 1 M KCl (lane 2), or 1 M KCl-5 M urea (lane 3); amounts of protein added were 9.5, 6, and 6 μ g, respectively; E and L, Positions of early and late transcripts, respectively; the sections of lanes 1 through 3 containing these transcripts were scanned densitometrically. Right: detection of T antigen by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transfer to nitrocellulose, and probing with anti-T antibody. The protein samples in lanes a, b, and c correspond to those added to transcription reactions 1, 2, and 3, respectively. The positions of protein molecular size markers on the gel are shown on the right in kilodaltons.

FIG. 4. Removal of T antigen from a 1 M KCl-5 M urea eluate of hydroxylapatite-bound SV40 chromosomes by using antibodies bound to *Staphylococcus aureus*. Left: effects on transcription. The hydroxylapatite (HAP) eluate (or as control dialysis buffer alone) was depleted of T antigen by treatment with antibody bound to *Staphylococcus aureus* (or mock depleted), as described in Materials and Methods. Transcription reactions were as shown in Fig. 1, the presence and absence of the hydroxylapatite eluate and anti-T antibody being indicated by + and -, respectively. E and L, Early and late transcripts, respectively. Right: detection of T antigen by immunoblotting as shown in Fig. 3. Samples loaded on the gel were as follows: hydroxylapatite eluate (lane a), intact SV40 chromosomes as marker for T antigen (lane b), and hydroxylapatite eluate depleted of T antigen, corresponding to that added to the transcription reaction in lane 3 (lane c). The protein molecular size markers are as described for Fig. 3.

HeLa cells that we used for transcription (Fig. 5, lanes 2 and 3). This extract protected against DNase I the 21-base-pair repeats, the origin-proximal part of each copy of the 72-base-pair repeat, and a region extending from position 240 towards the major late RNA start site. The region of the RNA start site, however, was not protected.

SV40 chromosomes were bound to hydroxylapatite, and the proteins were eluted with 1 M KCl-5 M urea (Fig. 1). The proteins in this eluate did not protect the 21- nor the 72-base-pair repeats (Fig. 5, lanes 4 and 5). They did protect a region between positions 240 and 300 and from 305 to 325, that is, between the 72-base-pair repeat and the major late RNA start site at position 325. Although both HeLa cellular and SV40 chromosomal proteins gave a footprint centered on position 270, several details of the pattern of bands around and within the protected region differ when the two footprints are compared. We conclude that the proteins from SV40 chromosomes which recognize this part of the SV40 late promoter are not the same as the HeLa cell proteins which also bind to this region.

The SV40 chromosomal proteins were eluted with 1 M KCl in the absence of urea. These proteins contained the late activating factor but not T antigen (Fig. 3). They also gave a DNase I footprint centered on position 270 (Fig. 5, lanes 6 and 7). (This protein fraction was apparently enriched for an exonuclease activity, since the shorter DNA fragments are shifted down in length by 1 or 2 nucleotides; however, this does not interfere with identification of the protected region.) By way of comparison, the three binding sites for purified T antigen on SV40 DNA are between nucleotides 5184 and 61 (for a review, see reference 15) in the region indicated in Fig. 5.

Gel mobility shift assay of protein binding to SV40 late promoter DNA. The method we used to isolate proteins from SV40 chromosomes extracted from infected CV-1 cells is not applicable to uninfected cells. To be able to compare DNA binding by proteins from infected and uninfected cells, nuclear extracts were prepared by the method of Dignam et

FIG. 5. Protection of SV40 origin-promoter region DNA against DNase I by proteins from HeLa cells and from SV40 chromosomes. The scale on the left shows the gel positions corresponding to SV40 nucleotide numbers. Lanes: M, size markers of SV40 DNA digested by *HinfI* with lengths of 237, 109, and 83 nucleotides; 1, labeled DNA probe (the *HpaII-HindIII* origin fragment recloned [see Materials and Methods]) digested with DNase I in the absence of added cellular extract; 2 and 3, DNase I protection by proteins in HeLa cell nuclear extract; the amounts of protein added were 20 µg (lane 2) and 40 µg (lane 3); 4 and 5, DNase I protection by proteins in a 1 M KCl-5 M urea eluate of hydroxylapatite-bound SV40 chromosomes; the amounts of protein added were 4.6 µg (lane 4) and 9.2 µg (lane 5); 6 and 7, protection by proteins in a 1 M KCl eluate of hydroxylapatite-bound SV40 chromosomes; 6, no added extract; 7, 7 µg of extract protein. The diagram on the right shows the positions of SV40 sequence landmarks, the region containing the origin of replication (ORI) and the T antigen (T ag) binding sites (15, 43; also see text), and the major late RNA start site.

al. (16). When mixed with a fragment of SV40 DNA spanning nucleotides 234 to 294, increasing amounts of uninfected-cell protein gave rise to several DNA-protein complex bands, retarded on gel electrophoresis (Fig. 6A). This result is thus consistent with the idea, mentioned above, that several proteins can interact with this region of SV40 DNA. Proteins from SV40-infected cells gave a different pattern. The faster-migrating bands formed with infected- or uninfected-cell protein comigrated, but the slowest-migrating band (the major band at high protein concentration) with infected-cell protein had a rate of migration quite distinct from the corresponding band formed with uninfected-cell protein. So the protein-DNA complexes containing infected-cell protein differ in their protein composition, or their shape, or both, from the complexes formed with protein from uninfected cells.

The formation of the retarded bands was reduced by the addition of an excess of the same DNA fragment, but unlabeled, to the binding reactions (Fig. 6B). The competitor poly(dI-dC) also reduced the formation of retarded bands by proteins from uninfected cells (Fig. 6C). However, poly(dI-dC) did not have the same effect on DNA-protein complexes containing infected-cell protein, and even at the highest concentration tested, the DNA remained in a slowly migrating complex (Fig. 6D). Therefore, the relative affinity of infected-cell proteins(s) for this DNA sequence is higher than that of uninfected-cell protein, at least as reflected in the ability of poly(dI-dC) to compete with binding.

FIG. 6. Gel mobility shift assays of the binding of proteins from SV40-infected and uninfected CV-1 cells to the *Bst*NI-*Kpn*I fragment of SV40 late promoter DNA. (A) Lanes: 1, no added protein; 2 through 4, 0.4, 0.8, and 4 μg of uninfected-cell protein (U), respectively; 5 through 7, 0.4, 0.8, and 4 μg of infected-cell protein (I), respectively. (B) Competition with unlabeled DNA fragment. Lanes: 1 through 3, standard binding reactions containing 2 μg of infected-cell protein and in addition 0, 10, and 50 ng of competitor DNA, respectively; 4 through 7, reactions containing 0.4 μg of infected-cell protein and 0, 1, 5, and 10 ng of competitor DNA, respectively. Formation of the retarded bands observed with uninfected-cell proteins was reduced by unlabeled DNA fragment in just the same way (data not shown). (C) Competition with poly(dI-dC). Lane 1, No added protein. The other binding reactions contained 2 μg of uninfected cell protein (U) and, in lanes 2 through 7, respectively, 0, 1, 5, 10, 50, and 100 ng of poly(dI-dC) competitor. (D) Competition as described for panel C except that the proteins were from SV40-infected cells. Lanes: 1, no added protein; 2 through 7, standard binding reactions with 2 μg of infected-cell protein (I) and 0, 1, 5, 10, 50 and 100 ng, respectively, of poly(dI-dC).

DISCUSSION

Progression of the SV40 lytic cycle to the late phase requires the early viral protein large T antigen (43). However, the LAF that we detected in this work does not appear to be T antigen. Although this result would seem unexpected, in fact it fits with several other observations. Temperature-shift experiments using temperature-sensitive mutants in T antigen (1, 14) and, more recently, using *ts*COS cells (38) first suggested that T antigen may be required transiently for establishment of late transcription but that once under way, late transcription can continue in its absence. We have shown (41) that the majority of SV40 chromosomes active in late transcription in vitro do not contain bound T antigen, while we confirmed that replicating chromosomes do (42). And finally, purified T antigen did not stimulate transcription from the late promoter in vitro, while it did inhibit the early promoter (36).

Nevertheless, we do not exclude that T antigen could exert a direct effect on late transcription, at least at certain RNA start sites. In the experiment whose results are shown in Fig. 3, the urea eluate, containing the T antigen as well as some LAF, was active in stimulating the late promoter. In a different approach to this problem, the role of the T antigen binding sites in controlling late transcription has been investigated by mutating them and analyzing promoter activity in

transfection experiments. The results suggested that T antigen can affect late transcription both indirectly and directly. Brady and Khoury (9) found that the T antigen binding sites at the origin play a role in the induction of late transcription. In these experiments, much of the RNA was initiated at sites other than the major late start site, and it was speculated that optimal activity of the major site may require additional transcription factors or replicated templates. Alwine and collaborators (2, 27) defined three sequence elements involved in activating the late promoter in the presence of T antigen. Omega, at the replication origin, and delta, between nucleotides 200 and 270, operated together. Tau, on the other hand, between nucleotides 150 and 200, functioned in the absence of T antigen binding sites. Thus, although there is an effect of the T antigen binding sites at the origin on late transcription, activation of the major late initiation site can occur without their being present. Of course, T antigen may interact with proteins as well as DNA (35).

In DNase I footprinting experiments, LAF preparations protected against digestion a DNA region centered on SV40 nucleotide position 270. Analysis of mutations affecting the late promoter show that this region is important for late promoter activity. Deletions between position 273 and 297 cut late promoter activity (17, 27), and the delta element of the late promoter (2, 27) overlaps the protected region. So the most straightforward interpretation of our results is that one and the same factor stimulated the late promoter in the transcription assay and gave the protection against DNase I in the footprinting assay.

Proteins in the HeLa cell nuclear extract also interacted with sequences around SV40 position 270 (Fig. 5) (45). This fits with the mutational analysis of the SV40 enhancer (46) which showed that deletions in this region reduced the effect of the enhancer on the SV40 early promoter on transfection into HeLa cells. The enhancer-binding proteins AP-1 and AP-4 have been reported to cooperate to give the base level of late transcription seen in vitro with HeLa proteins in the absence of LAF (34). However, LAF is different from these HeLa cell proteins because the footprint boundaries and sites cleaved within the overall protected regions are different. Recently, a late SV40 transcription factor present in whole-cell extracts of HeLa cells has been reported (28). This factor too does not seem to be the same as the factor LAF which we describe here because, apart from its coming from uninfected HeLa cells, the late SV40 transcription factor interacts with the 21-base-pair repeat element of the SV40 promoter region.

The SV40-coded capsid proteins could have an effect on the activity of the late promoter. The *ts*D class mutants of SV40, which have altered capsid proteins VP2 and VP3, are affected in control of transcription (4, 30, 32). However, the properties of *ts*D mutants suggest that the function of VP2 and VP3 is to inhibit SV40 transcription late in infection, not to stimulate it (32). Therefore, we think LAF is unlikely to be VP2 or VP3.

LAF therefore appears to be a cellular transcription factor which is induced by SV40 infection. Functional LAF activity was not found in uninfected cells. (41). Proteins from SV40-infected CV-1 cells formed a complex, retarded on gel electrophoresis, with a DNA fragment containing the LAF binding site. This complex had a higher stability and a different rate of gel migration than the complex formed with proteins from uninfected cells. Either LAF is newly synthesized in the infected cells or protein modification (3, 31) results in LAF from these cells having increased affinity for the DNA.

Although we describe LAF as a factor, it could comprise more than one protein. The footprints obtained with LAF preparations are large. The gel mobility shift assays indicate that a multiprotein complex can be formed on this stretch of DNA, and several factors from HeLa cells bind to overlapping sequences in the same region (34, 45). SV40 was recently reported (19) to alter the properties of CV-1 cell proteins which bind to the same recognition site as the HeLa cell transcription factor AP-1. AP-1 and another HeLa cell factor, AP-4, together recognize SV40 late promoter sequences (34). We now report that LAF preparations bind to the same DNA region as AP-4 and that the infected-cell proteins show increased stability of specific DNA binding. Together these observations suggest a model in which monkey cell proteins, related to these two HeLa cell factors, are induced by SV40 infection and then cooperate in activating viral late transcription.

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