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SV40 activates transcription from the transferrin receptor promoter by inducing a factor which binds to the CRE/AP-1 recognition sequence

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ABSTRACT

During the course of lytic infection by simian virus 40 (SV40), expression of both the viral late genes and certain host cellular genes is induced. The promoter of the cellular transferrin receptor (TR) gene contains a DNA sequence which is similar to the AP-1- and AP-4-binding region in SV40 which has been implicated in the control of the viral late promoter. Expression of TR is needed for cells to enter S-phase and is therefore expected to be important for the SV40 lytic cycle. Here we show that the level of TR mRNA *in vivo* was increased by SV40 infection. A factor which activates transcription from the TR promoter *in vitro* was specifically induced in SV40-infected cells. Gel mobility shift assays with an oligonucleotide comprising this part of the TR promoter showed three nucleoprotein complexes to be formed with proteins from CV-1 cells. Following SV40 infection, one of the complexes was increased ten-fold. Formation of this complex was specifically reduced by competition with the phorbol ester-responsive element of the collagenase gene, implying that the factor is a member of the AP-1/Jun/Fos family. Cross-linking of the complex by ultraviolet light showed major DNA-binding components to be proteins of about 55 kD and 47 kD. Removal of this factor by adding the oligonucleotide to *in vitro* transcription reactions with the TR promoter, abolished the activation of TR transcription. The factor which binds to the TR promoter co-sedimented with SV40 chromosomes extracted late in infection. This suggests that similar transcriptional regulatory proteins are involved in controlling transcription from both the SV40 and the TR promoters, and that the virus can use a common mechanism to induce viral and host cellular transcription.

INTRODUCTION

When simian virus 40 (SV40) infects permissive cells the expression of several host cellular genes is increased (1, reviewed in 2). This increase is due, directly or indirectly, to the SV40 early gene products the T antigens, and its function is believed to be to make the infected cell more efficient at replicating the virus. Increased expression of some cellular genes may also be important in cell transformation by SV40. SV40 infection raises the level of the mRNA of certain genes (3), including thymidine kinase (4), and the transcription factor Sp1 which in turn may stimulate transcription from other genes (5).

Also activated during the course of lytic infection by SV40 is the viral late promoter. This promoter is controlled by a number of proteins which interact with SV40 DNA. A HeLa cell protein, LSF, stimulates transcription from the SV40 late promoter *in vitro* (6). Two other HeLa proteins, AP-1 and AP-4, which are not related to LSF, act together to achieve the same result by binding to a site adjacent to the LSF binding site (7). In permissive monkey cells SV40 induces cellular factors, which could be related to AP-1 or TEF-1 (8, 9), and which are needed for late gene expression. We (10, 11) obtained, from isolated SV40 chromosomes, a late promoter activating factor preparation which functioned *in vitro* and which gave a DNaseI footprint covering the overlapping AP-1, AP-4, TEF-1 and LSF-binding sites of the late promoter.

A cellular gene whose expression is increased in cells infected or transformed by SV40 is the transferrin receptor (TR) gene. TR expression is necessary for cells to enter the S-phase of the cell cycle (12, 13). Since SV40 induces the transition to S-phase in infected cells, TR expression is expected to be important for SV40 replication. The promoter of the TR gene contains a TATA box, GC-rich regions, and a sequence similar to the cAMP- and phorbol ester-responsive elements (CRE and AP-1 sites) (14, 15, 16). This sequence binds two HeLa cell proteins, TREF-1 and 2 (15). The level of TR mRNA can also be shown to be regulated by iron concentration (16) by a post-transcriptional mechanism involving sequences at 3′ end of the gene (17).

Since the CRE or AP-1-binding region of the TR promoter has a similar DNA sequence to the AP-1 and AP-4-binding region of SV40 which is a controlling element in the viral late promoter, we were interested in testing whether related mechanisms operate to activate these two promoters in viral infection. In the work reported here we have looked at the regulatory proteins in uninfected cells, which interact with this region of the TR promoter and then at the changes which occur in the activity of these proteins upon infection of CV-1 cells with SV40.

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MATERIALS AND METHODS

DNAs

Cloned TR DNAs were a gift from Dr. L. Kühn. To prepare *in vitro* transcription templates, a clone derived from pcD-TR1 (16) containing the human TR promoter and upstream sequences to position −322 fused to TR cDNA sequences was digested by EcoRI and HindIII and the DNA fragment extending from −322 to the HindIII site at +890 isolated by agarose gel electrophoresis. A HindIII -HindIII fragment containing TR coding and 3′ non-coding sequences cloned in pSP64 vector was used as hybridization probe for TR mRNA.

The TR1 oligonucleotide comprised nucleotides −86 to −57 of the TR promoter and had the sequence: 5′-GTGCCTCAGG-AAGTGACGCACAGCCCCCCT annealed to its complement. The TRE oligonucleotide was a 20-mer containing the phorbol ester-responsive element of the human collagenase promoter (18). The HPV-18 AP-1 20-mer (19) was from the P104 promoter of human papillomavirus type 18 (HPV-18). The mutated form of this oligonucleotide had the GA of the AP-1 core changed to CT and no longer bound AP-1. The PTF-1 CD 37-mer was from the mouse alpha amylase promoter (20) and contained an AP-4 site. The Sp1 oligonucleotide was from the herpes simplex virus thymidine kinase promoter (21). KLT has a sequence from the mouse mammary tumour virus long terminal repeat, and has no known transcription factor binding sites (J. Billotte, unpublished). Oligonucleotides were labeled at the 5′ end using gamma-(^{32}P)ATP and T4 polynucleotide kinase before annealing with the complementary strands.

Proteins and SV40 chromosomes

Protein extracts of HeLa cell nuclei were prepared by the method of Dignam et al. (22) and contained 5−8 mg of protein per ml. Extracts of uninfected and SV40-infected CV-1 cells growing as monolayers were made by essentially the same method, slightly modified (10) and had a protein concentration of 6 mg/ml. SV40 chromosomes were isolated as previously described (23) from the nuclei of infected CV-1 cells 32 h post-infection, using the low ionic strength extraction buffer HBE (10 mM HEPES-KOH pH 7.9, 1 mM EDTA, 0.5 mM dithiothreitol). The SV40 chromosomes were further purified by sedimention through a gradient of 5% to 20% sucrose in HBE buffer in the Beckman SW60 rotor at 40,000 rpm for 75 min at 4°C. Fractions of 0.3 ml were collected from the bottom of the tube and stored at −70°C.

Gel mobility shift assays

Gel mobility shift assays contained, in 20 μl, 10 or 12 μl of Buffer D (Buffer D is 20 mM HEPES-KOH pH 7.9, 100 mM KCl, 20% glycerol, 0.2 mM EDTA, 0.5 mM dithiothreitol), 1 μg of poly (dI-dC), 25 fmoles of radioactively labeled duplex oligonucleotide and the protein to be tested. Where indicated the mix also contained 5 mM $MgCl_2$. The labeled oligonucleotide was added last. After 15 min incubation on ice the assay mix was loaded onto a 1.5% agarose gel 8 cm long containing 0.25×TBE buffer (24) and electrophoresed at 50 volts for 2 h at 4°C. The gels were dried and autoradiographed.

For ultraviolet light-induced cross-linking of proteins to DNA, after electophoresis the gels were wrapped in Saran and, lying on ice, were exposed to 254 nm light from an inverted transilluminator at 10 cm distance for 30 min. The protein-DNA complex bands were located by exposing the gel to X-ray film, the corresponding part of the gel cut out and the complexes recovered by slicing the gel and shaking overnight at 37°C in a solution containing 10 mM Tris-HCl pH 7.5, 100 mM NaCl and 1% sodium dodecylsulfate (SDS). Proteins were precipitated with 10 μg HeLa extract protein as carrier and 5 volumes of acetone overnight at −20°C. After centrifugation at 10,000×g for 20 min. at 4°C, the pellet was dissolved in sample buffer (24) and analysed by electrophoresis in a SDS, 10% polyacrylamide gel as described (24).

Transcription *in vitro*

In vitro transcription reactions containing 0.3 to 0.5 μg of the TR EcoRI/HindIII DNA fragment as template and HeLa cell nuclear extract were as previously described (23). The ^{32}P-labeled RNA was denatured with glyoxal and analysed by agarose gel electrophoresis (23).

RNA/DNA hybridization

Cytoplasmic RNA was isolated using phenol-chloroform extraction from uninfected CV-1 cells or from SV40-infected CV-1 cells at different times after infection. 15 μg-samples of RNA were spotted onto Hybond nylon membrane (Amersham) and fixed by ultraviolet irradiation as suggested by the maker. The membranes were prehybridised, then hybridised with a probe of TR HindIII DNA fragment (labeled by nick-translation) for 36 h at 42°C in 50% formamide, 5×Denhardt's solution, 5×SSC, 50 mM sodium phosphate pH 6.8, 0.1 mg/ml denatured salmon sperm DNA and 1% SDS (modified from ref. 24). After washing in 1×SSC, 1% SDS at room temperature then in 0.2×SSC, 0.2% SDS at 60°C, the membranes were autoradiographed.

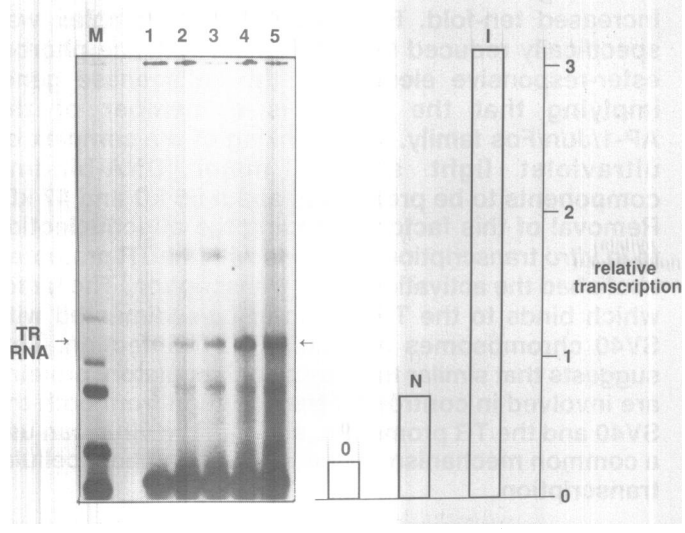

Figure 1. Transcription *in vitro* from the TR promoter. The reactions, containing as template the EcoRI-HindIII TR promoter DNA fragment and HeLa cell nuclear extract, were supplemented with 5 μl or 10 μl of the uninfected CV-1 (lanes 2 and 3) or SV40-infected CV-1 (lanes 4 and 5) extracts; lane 1: no supplement. Left panel: the 890-nucleotide TR run-off transcript is indicated; the transcript above it is an endogenous band associated with the CV-1 cell extracts. Lane M: size markers of SV40 DNA digested by HinfI. Right panel: the autoradiogram was scanned with a microdensitometer and the amount of transcript plotted in arbitrary units for lane 1 (O, no supplement), lane 2 (N, non-infected) and lane 4 (I, infected).

RESULTS

Transcription of the TR gene *in vitro*

The distance from the initiation site of TR mRNA synthesis to the HindIII site within the cDNA at which the template DNA was cleaved is 890 nucleotides (16). When this DNA was transcribed *in vitro* with a nuclear extract of HeLa cells, a single major run-off transcript of the expected length was seen (Fig. 1, lane 1 and Fig. 4, lane 1). To test whether SV40 induces a factor which stimulates the synthesis of the TR RNA, the transcription reactions were repeated with addition of increasing amounts of an extract of either uninfected (Fig. 1, lanes 2 and 3) or SV40-infected (Fig. 1, lanes 4 and 5) CV-1 cells. These additions had the result of increasing the activity of the TR promoter. For a given non-saturating amount of extract protein (30 μg), the uninfected extract stimulated the activity of the promoter by a factor of about 2.5 and the SV40-infected extract stimulated it by more than 10-fold (Fig. 1, lanes 2 and 4, and left panel). We conclude that a factor needed for efficient TR transcription is limiting in the HeLa cell extract, that it is present in the CV-1 extract and in higher amounts in the extract of SV40-infected CV-1 cells.

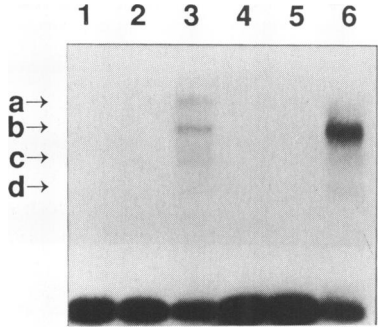

Figure 2. Gel mobility shift assay of protein binding to the TR1 oligonucleotide. Labeled TR1 was incubated without protein (lanes 1 and 4) or with uninfected CV-1 cell extract protein (lanes 2 and 3) or SV40-infected CV-1 extract protein (lanes 5 and 6). Amounts of extract: 0.2 μl (lanes 2 and 5) or 2 μl (lanes 3 and 6). The nucleoprotein complex bands are designated *a*, *b*, *c* and *d*. Free labeled oligonucleotide is at the bottom of the gel.

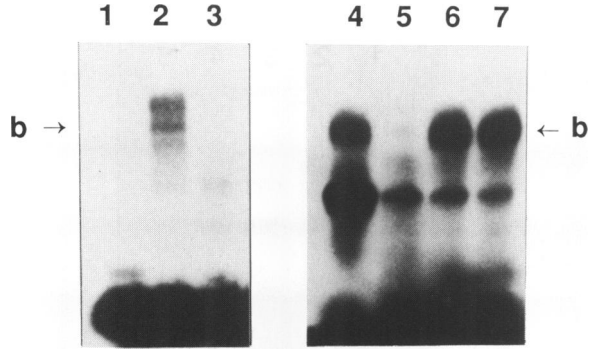

Figure 3. Specificity of nucleoprotein complex formation with TR1 oligonucleotide. Nucleoprotein complexes were formed with 1 μl HeLa cell extract (lanes 2 and 3) or with 3 μl of an SV40 chromosome preparation (lanes 4–7). Competing unlabeled oligonucleotides present at a 10-fold molar excess were: lane 3, TR1; lane 5, collagenase TRE; lane 6, mutated HPV-18 AP-1 sequence; lane 7, the unrelated oligonucleotide KLT. The position of the nucleoprotein complex *b* is indicated.

Gel mobility shift analysis of the interaction of proteins with the TR transcriptional control element

The TR transcriptional control element has been located about 70 base-pairs upstream of the TR mRNA start site (14, 15). This element contains the sequence related to the CREB and AP-1 recognition sequences. We synthesised an oligonucleotide, TR1 (see Materials and Methods) which included this element and the sequences which surround it. In gel mobility shift assays with uninfected CV-1 cell protein extracts, four protein-DNA complexes (named *a*–*d*) were detected (Fig. 2, lane 3). With HeLa cell extracts the same complexes were seen (not shown here, but see Fig. 3). When SV40-infected CV-1 cell extracts were tested, formation of one of the complexes (band *b*) was stimulated more than 10-fold while that of the others was not affected (Fig. 2, lane 6). Fig. 3 shows the effect of competition by unlabeled oligonucleotides on TR1 nucleoprotein complex formation with proteins from, in lanes 1–3, a HeLa extract or, in lanes 4–7, an SV40 chromosome preparation (see Fig. 6). Formation of complexes *a*, *b* and *c* was abolished by competition with the same oligonucleotide but unlabeled (Fig. 3, lanes 2 and 3), but not by an unrelated oligonucleotide KLT (Fig. 3, lane 7, data for HeLa not shown) nor by a mutated HPV-18 AP-1 sequence (Fig. 3, lane 6). Band *b* was reduced by competition with a wild type AP-1 element (Fig. 3, lane 5); this will be discussed in more detail below.

DNA sequences in the TR1 oligonucleotide are important for activation of the TR promoter *in vitro*

The TR promoter-containing DNA fragment was transcribed *in vitro* in the absence or presence of SV40-infected CV-1 cell extract (Fig. 4, lanes 1 and 2 respectively) or with, in addition, a 10-fold molar excess of the TR1 oligonucleotide (Fig. 4, lane

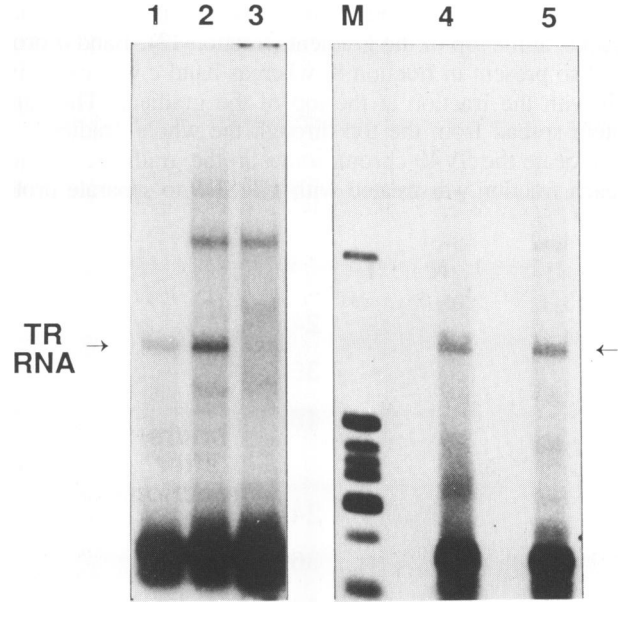

Figure 4. The effect of competing oligonucleotides on transcription from the TR promoter. Transcription reactions as in Fig. 1 contained HeLa cell extract (lane 1) or in addition 7 μl of extract from SV40-infected CV-1 cells (lanes 2–5). Competing oligonucleotides were in 10-fold molar excess over the template and were TR1 (lane 3) or the pSP65 polylinker (lane 5); they were added to the protein extracts for 10 min at 0°C before the start of the transcription reactions. Lane M: marker of pBR322 digested with HinfI.

3). The presence of the TR1 oligonucleotide abolished specifically the activation of TR transcription, even a basal level being barely detectable (compare Fig. 4, lanes 2 and 3). Addition of an unrelated oligonucleotide (Fig. 4, lanes 4 and 5) had no effect on TR transcription. We conclude from this competition experiment that protein binding to TR promoter DNA sequences that are included in the TR1 oligonucleotide is important for promoter activity.

Infection by SV40 increases the level of cytoplasmic TR RNA

Cytoplasmic RNA was isolated from CV-1 cells at different times after infection by SV40, or mock-infection without virus. Aliquots were applied to nylon membranes and the TR-specific RNA measured by hybridisation with a probe comprising TR coding and 3′ non-coding sequences (Fig. 5). The uninfected cell RNA already contained easily detectable amounts of TR RNA and these remained constant throughout the time-course of the experiment (Fig. 5, samples in the column marked N). After SV40 infection the amounts of TR RNA followed a reproducible pattern : the amount increased to a maximum at 36 h post-infection and by 48 h had fallen again to a level below that in RNA from uninfected cells (Fig. 5, column marked I). At 48 h the infected cells were showing signs of virus-induced cytopathic effect and this probably explains the falling amounts of TR RNA.

The factor which forms the major complex with TR1 oligonucleotide co-sediments with SV40 chromosomes

SV40 chromosomes were extracted from CV-1 cells 32 h after infection, then analysed by sedimentation in a 5% to 20% sucrose gradient. The gradient fractions were tested, using the gel mobility shift assay, to detect the proteins which form complexes with the TR1 oligonucleotide. The result (Fig. 6, upper panel) showed that the different proteins had quite distinct sedimentation behaviour. The protein responsible for the major band (*b*) formed a peak in fractions 7 and 8, and was also present in smaller amounts at the top of the gradient (fraction 12). Band *a* protein was also present in fraction 8, whereas band *c* was exclusively seen with the fraction at the top of the gradient. The band *d* protein spread from the top through the whole gradient.

To locate the SV40 chromosomes in the gradient, an aliquot of each fraction was treated with 1% SDS to separate proteins from DNA, and the DNA analysed by agarose gel electrophoresis (Fig. 6, lower panel). The supercoiled and relaxed forms of SV40 DNA were found mostly in fractions 7 and 8, the same fractions that contained the band *b* (and band *a*) proteins. The proteins forming bands *a* and *b* therefore co-sediment with SV40 chromosomes while those forming bands *c* and *d* do not. The band *d* protein, on the basis of these results and competition experiments (Fig. 3, lanes 4−7) seems not specific for the DNAs we tested.

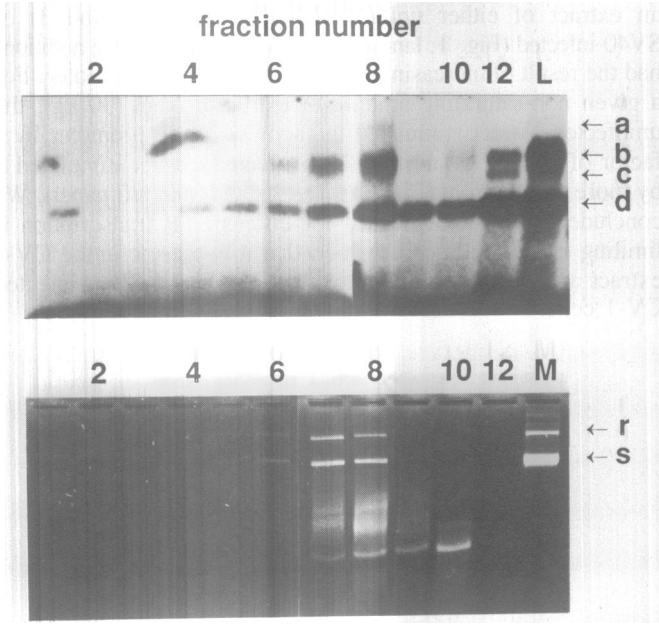

Figure 6. Factors which recognise the TR promoter are extracted from SV40-infected cells and co-sediment with SV40 chromosomes. SV40 chromosomes were centrifuged in a sucrose gradient as in Materials and Methods. Sedimentation is from right to left. Upper panel: aliquots (7 μl) of the gradient fractions were tested by gel mobility shift assay for proteins binding to the TR1 oligonucleotide. The positions of complexes *a* to *d* (see Fig. 2) are indicated. Sample L: the preparation of SV40 chromosomes before loading on the sucrose gradient. Lower panel: gradient aliquots (5 μl) were deproteinised and the DNA analysed by agarose gel electrophoresis and ethidium bromide staining. Lane M: marker of SV40 DNA; the positions of the supercoiled (s) and relaxed (r) circular forms of SV40 DNA are shown.

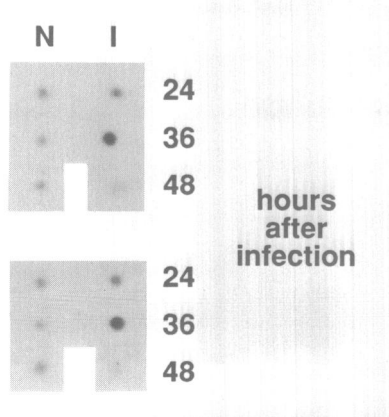

Figure 5. Hybridisation analysis of TR mRNA in non-infected and SV40-infected CV-1 cells. Cytoplasmic RNA was extracted from CV-1 cells 24, 36 or 48 h after infection by SV40 or at the same times after mock-infection. 15 μg aliquots were fixed to nylon membranes and hybridised with a labeled TR sequence-specific DNA probe. N, non-infected cell RNA; I, infected cell RNA. The upper and lower panels show the results of two experiments.

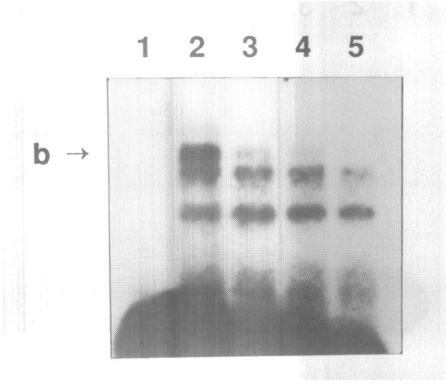

Figure 7. Effect of competition by the phorbol ester-responsive element on formation of nucleoprotein complexes with TR promoter DNA sequences. Gel mobility shift assays contained labeled TR1 oligonucleotide and, in lanes 2 to 5, 3 μl of SV40 chromosome preparation (see Fig. 6, lane L). Lanes 3 to 5: 4-, 8- and 16-fold molar excess respectively of collagenase TRE oligonucleotide was added before the labeled probe. The position of complex *b* is shown.

The formation of TR1 nucleoprotein complex *b* is specifically reduced by competition with an oligonucleotide containing a phorbol ester-responsive element

We tested the affect on protein binding to the TR1 oligonucleotide, of adding competing oligonucleotides with known binding specifities. The TR1 oligonucleotide contains a sequence homologous to the closely related cyclic AMP-responsive (CRE) and phorbol ester-responsive (AP-1) elements (15). Formation of the major band *b* complex was efficiently and specifically reduced by an oligonucleotide containing the phorbol ester-responsive element (18) of the human collagenase promoter (Fig. 7, see also Fig. 3, lane 5). Band *b* was likewise reduced by the AP-1 sequence of the HPV-18 promoter but not by a mutated sequence which no longer binds AP-1 (Fig. 3, lane 6). On the basis of this binding specificity, band *b* is formed, apparently, by a protein related to the AP-1/Jun/Fos or CREB/ATF/ families (25, 26).

One of the factors reported to be important in activating the SV40 late promoter is AP-4 (7) and the TR1 oligonucleotide contains, adjacent to the CRE-related sequence, a sequence homologous to part of the AP-4 binding site in SV40. Competition experiments were done to test the effect of adding the oligonucleotide PTF-1 CD (which is from the mouse alpha amylase promoter and contains a canonical AP-4 site, refs 20, 27); however, no effect on any of the TR1 protein-DNA complexes was seen (not shown). In the same way an oligonucleotide with the sequence of part of the herpes simplex virus thymidine kinase promoter containing a binding site for the factor Sp1 (21) was tested. This oligonucleotide did not affect band *b* but specifically reduced the formation of band *a* (not shown). Therefore band *a* may consist of factor Sp1 (or another factor which recognises such GC-rich sequences) bound to the TR1 oligonucleotide.

The TR1 nucleoprotein complex *b* contains proteins of about 55 kD and 47 kD

To estimate the size of the protein which, together with the TR1 oligonucleotide, forms complex *b*, a scaled-up gel mobility shift assay was done with proteins from SV40-infected CV-1 cells. The gel was irradiated with ultraviolet light to cross-link proteins and DNA, band *b* was located by autoradiography and the complexes eluted and recovered. These were then analysed by SDS polyacrylamide gel eletrophoresis with protein molecular weight standards. The migration of the major band seen corresponded to a size for the complex of 65 kD which, after deducting the molecular weight (9.8 kD) of the oligonucleotide yields an estimated molecular weight for the protein of 55 kD. Two additional bands on the gel corresponded to proteins of 47 kD and 30 kD. When the same experiment was done with proteins from HeLa cells the protein components of complex *b* had similar sizes to those found with SV40-infected CV-1 cell proteins.

DISCUSSION

We have shown that a transcription factor induced by SV40 stimulates transcription from the TR promoter *in vitro*. SV40 also induces a single strong band in the gel mobility shift assay with an oligonucleotide containing the TR transcriptional control element. When the reactions were depleted of the protein binding this oligonucleotide, transcriptional stimulation of TR was no longer seen. This suggests that SV40 induces TR transcription by way of changes in transcriptional regulatory proteins which bind to the TR transcriptional control element.

Why should SV40 induce TR expression ? An important function in the SV40 lytic cycle is the switch which stimulates resting cells to enter the S-phase of the cell cycle (2). In this way the virus ensures that cellular DNA replication enzymes, which the virus needs for its own replication, are available. Transformation of cells can occur if the lytic cycle aborts while there is continued action of the stimulus to progress in the cell cycle. The expression of TR has been shown to be needed for cells to enter S-phase (12, 13). TR expression is therefore expected to be important for efficient viral growth, and possibly in transformation. Transformed cells do express high levels of TR (reviewed in 15).

How does SV40 induce TR expression? What is the factor which forms the SV40-induced complex *b*? The transcriptional control element of the TR promoter contains the sequence

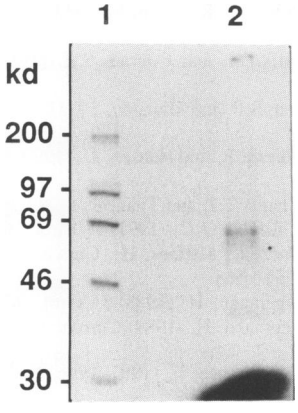

Figure 8. Ultraviolet light-induced cross-linking of proteins from SV40 chromosomes, to the TR1 oligonucleotide. A gel mobility shift assay was done with an SV40 chromosome preparation from infected CV-1 cells and TR1 DNA. The gel was exposed to ultraviolet light, the complex *b* recovered and analysed by SDS polyacrylamide gel electrophoresis. Lane 1, ^{14}C-labeled protein molecular weight standards (Amersham); lane 2, cross-linked products. Free oligonucleotide is at the bottom of the gel.

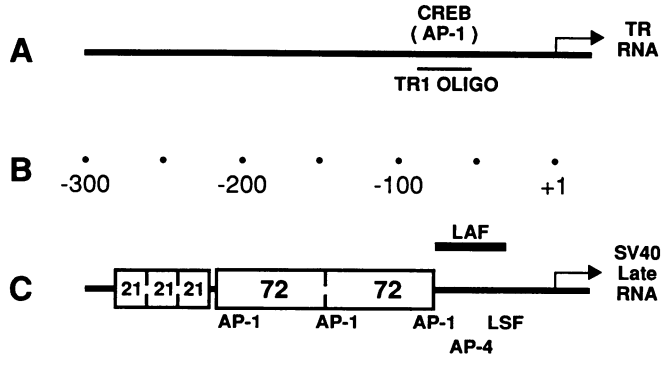

Figure 9. The positions of AP-1 or AP-1-like sequences in the TR promoter and SV40 late promoter. A: the TR promoter region showing the relative positions of the RNA start site, the CREB (AP-1) binding site and the TR1 oligonucleotide. B: scale of nucleotide positions relative to the RNA start sites, valid for the TR and SV40 sequences. C: the SV40 late promoter region showing the 21-base pair and 72 base-pair repeats, binding sites for certain proteins and the region protected against DNase I by the late activating factor (LAF) preparation from SV40 chromosomes (10, 11).

5'-GTGACGCAC which is related to, but not identical with, the CREB/ATF binding site central motif GTGACGTCA, and that of AP-1 GTGACTCA. The major nucleoprotein complex (complex *b*) formed with the TR1 oligonucleotide and proteins from SV40-infected cells was reduced specifically by competition with the phorbol ester-responsive element of the collagenase gene which contains a canonical AP-1 binding site. Complex *b* was not affected by oligonucleotides containing the binding sequences of either the proteins AP-4 or Sp1. This suggests that the protein forming complex *b* is a member of the family of proteins which recognise AP-1- or CRE-related sequences and which includes the Jun, Fos, CREB and ATF proteins.

Proteins affinity-purified using the AP-1- or ATF-binding sequences form a heterogeneous set (25, 28, 29, reviewed in 30), with sizes ranging from 39 kD (c-Jun) to 55–60 kD (c-Fos). The major protein component of the SV40-induced complex *b* which could be cross-linked to DNA with ultraviolet light had an apparent size (55 kD) not significantly different from that of c-Fos. The 47 kD polypeptide had the same size as CREB-related proteins (25) and Jun D (32), when observed by ultraviolet light cross-linking. Fos is cross-linked to DNA more efficiently than Jun (32). In our competition experiments the band *b* protein showed a high affinity for the canonical AP-1 recognition sequence. Therefore we think it likely that the formation of the nucleoprotein complex *b* and the stimulation of TR transcription was due to a dimer of Fos and a Jun- or CREB-related protein binding to the CRE/AP-1 recognition sequence of the TR promotion.

AP-4 has been suggested to play a role in SV40 late transcription (7) and there is a sequence partly homologous the AP-4 recognition site immediately adjacent to the AP-1 site in the TR promoter (the same position as the AP-4 site in SV40, ref. 7). Nevertheless, we found no evidence that AP-4 is involved in the stimulation of transcription of TR: competition with the PTF-1 CD oligonucleotide which contains the canonical AP-4 sequence CAGCTG did not reduce protein binding to the TR1 oligonucleotide; and the amount of AP-4 (detected by nucleoprotein complex formation with PTF-1 CD) was not increased by SV40 infection (unpublished results). The factor TEF-1 has also been implicated in SV40 late transcription (8). TEF-1 binding sequences (9) are not present, however, in oligonucleotides that the complex *b* protein binds to.

Aside from the effects of SV40 infection described here, expression of the TR gene is controlled by the cell's need for iron and as a function of cell growth or differentiation (15, 16). TR expression is increased by other mitogens, and the TR promoter has been reported (15) to be induced several-fold by stimulation of cell growth. The TR transcriptional control element is needed for maximum activity of the TR promoter in HeLa cells (14). Since AP-1 activity is stimulated by serum (30), the mechanism of regulation of the TR promoter in growing cells may be related to the mechanism of its induction by SV40 infection that we describe. However, the HeLa cell proteins found by Roberts et al. (15) to bind to the TR transcriptional control element, TREF1 (83 kD) and TREF2 (62 kD), have different molecular weights from the SV40-induced factors(s) we found. The TR promoter contains several GC-rich regions with the sequence of Sp1 binding sites (14). The TR1 oligonucleotide does not contain any of these canonical Sp1 binding sequences, but it does contain a GC-rich stretch (see Materials and Methods). The gel mobility shift band *a* that we observed was specifically reduced by competition with DNA containing a strong Sp1 site. Band *a* may therefore correspond to Sp1 binding to the TR1 oligonucleotide. In any case, it was not apparently induced by SV40 infection.

Is there a link between the ways in which SV40 late transcription and expression of the TR gene are induced? In Fig. 9 the TR and SV40 late promoters are aligned for comparison (only certain transcription factor binding sites are shown). SV40 late transcription is controlled by a combination of trans-acting factors and chromatin structure (23). One of the factors which has been implicated in activiting late SV40 transcription is AP-1, perhaps in combination with AP-4 (7). AP-1 binding regions in SV40 DNA are needed for late gene expression and viral-induced changes in the proteins recognising these regions of SV40 DNA have been reported (8 and references therein, 31). The late activating factor preparation from SV40 chromosomes (10, 11) gave a broad DNase I footprint which included the late promoter-proximal AP-1 and AP-4 sites (see Fig. 9), and it stimulated transcription *in vitro* from the viral late promoter. It is probable, then, that the induced AP-1 activity is interacting with AP-1 sites in both the SV40 late and TR promoters, and therefore that the virus uses a common mechanism to activate viral and cellular gene expression. How this factor is induced, whether its induction involves new synthesis or modification of existing protein, are questions for further work.

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