SV40 stimulates expression of the *trans*-acting factor Sp1 at the mRNA level

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Expression of the *trans*-acting transcription factor Sp1 increased almost 10-fold after infection of cells by simian virus 40. This alteration, attributable to an early viral protein, occurred at the mRNA level beginning at 12 hr postinfection, shortly after the appearance of viral T antigen, and reached a plateau at 20 hr postinfection. The enhanced level of Sp1 message was accompanied by a marked increase in Sp1 protein in the cell nuclei. Furthermore, we have demonstrated that stimulation of Sp1 levels elevates expression from viral and cellular promoters. Enhancing the amount of this *trans*-acting factor may play a role in aiding the viral life cycle and in neoplastic transformation of infected cells.

[*Key Words*: Sp1; SV40; T antigen]

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T antigen, the oncogene product from simian virus 40 (SV40), has pleiotropic effects that contribute to the viral life cycle and to neoplastic transformation (Livingston and Bradley 1987; Stahl and Knippers 1987). For both processes, T antigen alters normal host cell regulation. For example, T antigen stimulates cellular DNA synthesis (Chou and Martin 1975) and causes G_1-arrested cells to enter S phase (Soprano et al. 1979). The viral oncogene product also affects the steady-state levels of cellular RNA species (Shutzbank et al. 1982) including thymidine kinase (Stuart et al. 1985; Stewart et al. 1987), and enhances rRNA synthesis by RNA polymerase I (Soprano et al. 1979). To define specific events in the transformation process, it is important to understand the mechanisms by which T antigen induces these cellular regulatory changes.

T antigen may accomplish its diverse effects through interactions with tumor suppressor proteins. It has been known for some time that the cellular protein p53 is associated with T antigen, and recent work suggests that this association extends to all structural subclasses of T antigen (Montenarh et al. 1986). Although p53 was once thought to have a direct role in cell transformation (Eliyahu et al. 1984; Parada et al. 1984), only mutated forms have an oncogenic capacity (Finlay et al. 1988; Hinds et al. 1989), and the native p53 appears to be a tumor suppressor protein (Baker et al. 1989). T antigen is also associated with the product of a recessive oncogene, the retinoblastoma (*Rb*) susceptibility gene (DeCaprio et al. 1988; Ludlow et al. 1989). This observation is of great significance as the interaction between T antigen and the *Rb* product is dependent on the T-antigen domain required for transformation.

Modulation of the activity of cellular *trans*-acting factors is another mechanism by which T antigen alters cellular regulation. The cellular factor AP-2 is inactivated by T antigen through direct interaction (Mitchell et al. 1987). T antigen also induces a SV40 late promoter-activating factor, which is not found in uninfected cells (Beard and Bruggmann 1988). Another cellular transcription factor, possibly AP-1, is induced or modified by T antigen to stimulate viral late gene expression (Gallo et al. 1988).

Although these observations could explain some or all of the effects of T antigen on cellular regulation, we hypothesized that there would be additional, perhaps redundant, pathways for altering cellular regulation. Because of the examples of T antigen altering the activity of transcription factors, we have examined the effect of viral infection on another *trans*-acting factor, Sp1. Sp1 is of particular interest because it has a critical role in transcription of both viral and cellular genes.

Sp1 is a 95- to 105-kD protein expressed in all mammalian cells (Briggs et al. 1986). This factor binds to the GC-box motif found in the 21-bp repeats of the SV40 regulatory region and is required for viral transcription (Dynan and Tjian 1983a,b). Promoters from the herpes simplex virus (HSV-1; Jones and Tjian 1985) and the human immunodeficiency virus (HIV-1; Jones et al. 1986) also utilize Sp1. Sp1 is required for transcription of the cellular genes dihydrofolate reductase (Dynan et al. 1986) and the Harvey *ras1* proto-oncogene (Ishii et al. 1986). Many other cellular genes, including DNA polymerase β (Yamaguchi et al. 1988), HMG coenzyme A (CoA) reductase (Reynolds et al. 1984), adenosine deaminase (Valerio et al. 1985), and hypoxanthine phosphori-

bosyltransferase (Melton et al. 1986), contain one or more GC-box motifs within their promoter sequences and may therefore interact with Sp1.

Given the widespread role of Sp1 in regulating transcription of viral and cellular genes, we considered Sp1 to be a likely target for T-antigen induction. We have found that infection of cells with SV40 results in an almost 10-fold increase in the amount of Sp1 mRNA, which is reflected at the protein level. This activation by SV40 infection represents a useful viral strategy for altering the host cell for the benefit of the viral life cycle and possibly neoplastic transformation.

Results

SV40 infection stimulates Sp1 expression at the mRNA level

Monkey kidney fibroblast cells (CV-1) were infected with SV40 (strain 776) at a multiplicity of infection (MOI) of 5. Because viral infection stimulates cell growth, we used cells at low density (1.3×10^4 cells/cm^2) so that all cells continued logarithmic growth during the experiment. In this way, observed changes are more likely to be a direct influence of viral infection than an indirect effect from stimulating confluent cells to re-enter the cell cycle. At various times after infection, total RNA was isolated from the infected cells and the level of Sp1-specific message was quantitated by Northern blot analysis. After electrophoresis and transfer to a nylon membrane, blot-shadowing (Thurston and Saffer 1989) was used to confirm that equal amounts of RNA from each time point were fixed to the filter (Fig. 1A). Hybridization to the Sp1 cDNA (pSp1-1; Kadonaga et al. 1987) detected the expected 8.2-kb message and showed an induction by SV40 (Fig. 1B). The amount of Sp1 message began to increase 12 hr postinfection, shortly after T-antigen production began (Acheson 1981). Sp1 mRNA reached a maximum by 20 hr (Fig. 1B), and no further change occurred for up to 36 hr (data not shown). We have also carried out experiments at the higher cell density of 7×10^4 cells/cm^2. Under these conditions, SV40 induced a similar change in Sp1 mRNA levels (data not shown).

In addition to using equal amounts of total RNA from the infected and uninfected cells, we have used an internal negative control, β-2 microglobulin, whose expression is unaffected by SV40 infection (Stuart et al. 1985; Stewart et al. 1987). The level of β-2 microglobulin mRNA remained constant over the course of the experiment (Fig. 1B), which further ensures that the rise in Sp1 message is not due to differences in the amount of RNA on the gel. Densitometric analysis of the data in Figure 1B and from other experiments (not shown) demonstrated that the amount of Sp1 message, relative to the internal β-2 microglobulin control, increased 8.5-fold (range, 7- to 10-fold) after SV40 infection.

As a positive control, we have probed the RNA from the infected cells for thymidine kinase mRNA, which is stimulated 10- to 20-fold by SV40 infection of confluent cells (Stuart et al. 1985; Stewart et al. 1987). We confirmed the SV40 induction of this message but observed only a fourfold increase in our experiments (Fig. 1B). This smaller effect was probably due to the different cell densities and, hence, growth state, used.

The Sp1 mRNA increase is reflected at the protein level

To determine whether higher Sp1 mRNA levels result in greater expression of the Sp1 protein, we employed immunofluorescence microscopy. Cells from the same SV40 infection used in the experiment shown in Figure 1 were stained for either Sp1 or T antigen by use of antibodies specific for those proteins (Fig. 2). Under the conditions used, Sp1-specific immunofluorescence was barely detectable in uninfected cells (Fig. 2). However, beginning at 16 hr postinfection, the amount of Sp1-specific nuclear fluorescence intensified, reaching a maximum at ~20 hr. For comparison, other cells from this experiment were stained for T antigen, whose production was just detectable in nuclei by 16 hr and continued to rise through 24 hr postinfection (Fig. 2).

In addition to these experiments, we have used double staining to correlate T-antigen production and Sp1 syn-

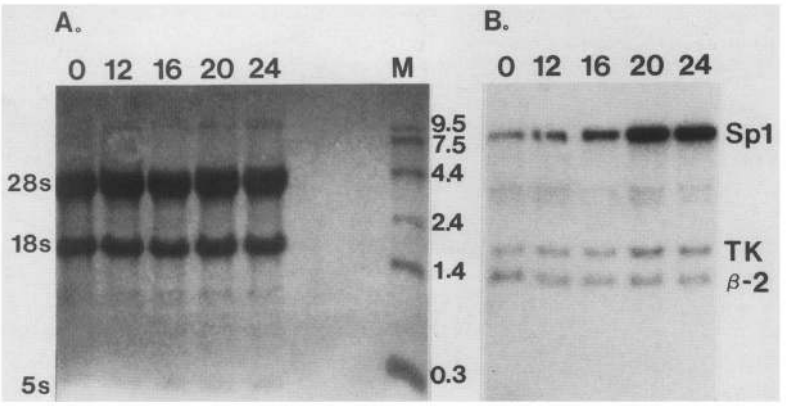

Figure 1. Stimulation of Sp1 message by viral infection. CV-1 cells were infected with SV40 (strain 776) as described in Materials and methods. Total RNA was isolated at the times (hr) indicated at *top*, and equal amounts (5 μg) were separated on a 1.2% agarose/2.2 M formaldehyde gel, followed by transfer to a nylon membrane. (*A*) Blot-shadowing of the filters (Thurston and Saffer 1989) was used to demonstrate that equal amounts of RNA were transferred. The positions of the rRNAs are indicated at *left*; sizes (in kb) of the RNA markers (lane M) are shown at *right*. (*B*) The autoradiograph of the filter after hybridization to human cDNA probes for Sp1, β-2 microglobulin (β-2), and thymidine kinase (TK) is shown at the same scale as the filter in *A*. The bands resulting from each probe are indicated at *right*. The faint doublet at ~4 kb is due to Sp1 hybridization but is otherwise unidentified.

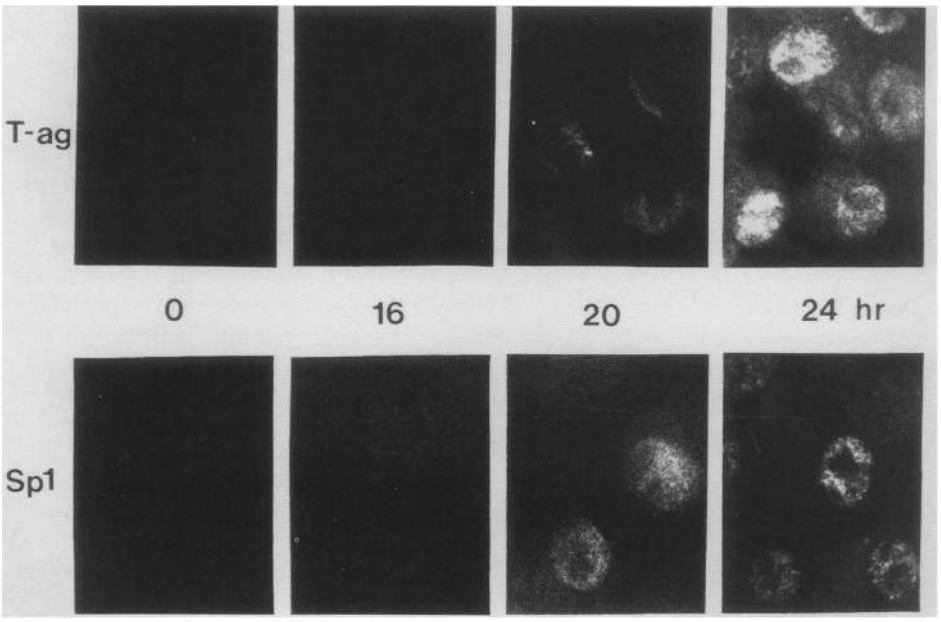

Figure 2. Immunofluorescence of Sp1 and T antigen in SV40-infected CV-1 cells. Cells from the same experiment shown in Fig. 1 were used for immunofluorescent detection of Sp1 and T antigen (T-ag). Using an Sp1-specific polyclonal antibody or the anti-T antigen monoclonal, pAB419 (Harlow et al. 1981), cells were stained as described in Materials and methods. The times postinfection are shown between the two rows. The bright staining is localized in the nuclei.

thesis. In particular, nuclei failing to display enhanced Sp1 staining also did not stain for T antigen and, thus, were not infected (data not shown). These data show that SV40 infection of CV-1 cells caused an increase of Sp1 protein.

Although immunofluorescence is not quantitative, the remarkable change in Sp1-specific staining in infected cells indicates at least a 10-fold increase in the protein level. Further experiments are required to quantitate accurately the change in Sp1 protein level, as well as to investigate any post-translational or functional differences.

An SV40 early gene is responsible for the increased expression of Sp1

Because it is known to be responsible for many changes in gene expression (Livingston and Bradley 1987; Stahl and Knippers 1987), we hypothesized that T antigen was responsible for the elevated expression of Sp1. To test this, CV-1 cells were transfected with a plasmid containing only the early region of SV40, pSVE (see Materials and methods), and grown for 72 hr. The levels of Sp1 and T-antigen expression in the transfected cells were assessed with immunofluorescence. Cells were simultaneously stained with the mouse monoclonal pAb419 (Harlow et al. 1981) to detect T antigen and a rabbit polyclonal anti-Sp1 to detect Sp1. The second antibodies used were fluorescein-conjugated goat anti-mouse IgG and rhodamine-conjugated goat anti-rabbit IgG.

Staining for T antigen was seen in ~10% of the cells, indicating the transfection efficiency. Two cells are present in the field shown (Fig. 3). At the top, T-antigen expression was detected in only one cell. Below, Sp1 was found in both cells but at a substantially higher level in the cell expressing T antigen. This experiment showed that the expression of the viral early gene products alone was capable of stimulating Sp1 expression. Identical results were seen in control transfections using a plasmid, pSV40, containing the entire viral genome. In both experiments, we did note, however, that a fraction of the cells staining positive for T antigen did not have elevated Sp1 (data not shown). The reason for this is being investigated further.

An increase in Sp1 level stimulates expression from Sp1-responsive promoters

To determine whether the higher Sp1 protein level contributes to the viral life cycle, we have directly augmented the Sp1 level in cells. A vector, pSVSp1-F (Fig. 4), was made with the SV40 early promoter driving expression of the human Sp1 cDNA from pSp1-1 (Kadonaga et al. 1987). The choice of promoter was, in part, based on the assumption that overexpression of Sp1 would contribute to higher activity from the Sp1-responsive SV40 promoter. Although the Sp1 cDNA used does not encode the extreme amino terminus of wild-type Sp1, the truncated Sp1 protein produced is fully functional (Courey and Tjian 1988; Kadonaga et al. 1986). The ability of the vector to overexpress Sp1 was confirmed at the RNA level.

The reporter vector pSV2CAT (Gorman et al. 1982a), which expresses chloramphenicol acetyltransferase (CAT) under the control of the SV40 early promoter, was

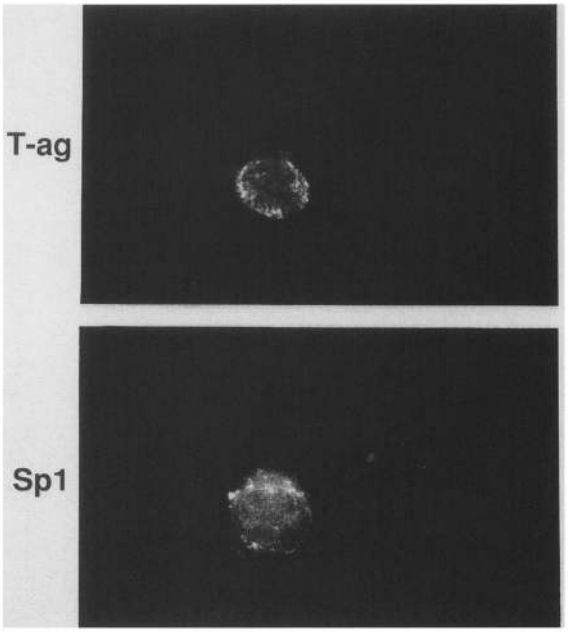

Figure 3. The enhanced expression of Sp1 is due to an SV40 early protein. CV-1 cells were transfected with the plasmid pSVE, which expresses the viral early proteins. After 72 hr, cells were harvested and the expression of T antigen and Sp1 was detected by immunofluorescence. (*Top*) A field containing two cells was examined for fluorescein-specific fluorescence which is indicative of T-antigen expression. (*Bottom*) The same field was examined for rhodamine-specific fluorescence, which is indicative of Sp1 expression.

cotransfected into COS-1 cells (Gluzman 1981) with pSVSp1-F or the control vector, pSV2A101 (Fig. 4). When transfected into these cells, both the reporter CAT vector and the Sp1 expression vector replicate to a high copy number comparable to that of the replicating viral genome in SV40-infected cells (data not shown). With the high copy number of the SV40 regulatory sequences and the expression of T antigen, the COS-1 cells approximate the environment of SV40-infected cells. The CAT activity in the transfected cells overexpressing Sp1 was greatly stimulated as compared to the control cells (Fig. 5).

To get a more general view of the effects of altered Sp1 levels in normal cells, we carried out a similar experiment with CV-1 cells. As in COS-1 cells, overexpression of Sp1 increased CAT activity from the pSV2CAT reporter vector (Fig. 6). These changes in CAT activity occurred at the RNA level, as determined by analysis of CAT mRNA (data not shown).

In addition, a CAT vector with the mouse glycerol phosphate dehydrogenase (GPDH) promoter was tested to define the effect of higher Sp1 levels on a typical cellular promoter. Expression from the GPDH promoter, which contains a single GC box, is also increased by overexpression of Sp1 (Fig. 6). As mentioned above, the increased CAT activity has been shown to occur at the RNA level (data not shown).

We also examined the effect of overexpression of Sp1 on the human β-globin promoter, which does not contain a GC box. A vector using this promoter to drive expression of the CAT gene pβCAT was transfected into CV-1 cells along with pSV2A101 or pSVSp1-F. Surprisingly, expression from this promoter was also enhanced by overexpression of Sp1 (Fig. 7), although not to the same extent as for the SV40 or GPDH promoters (see legend to Fig. 6). In light of this finding, we made a construct analogous to pSVSp1-F in which an 8-bp *Xho*I linker was inserted at the beginning of the Sp1 gene to create a frameshift mutation. This plasmid, termed pSVSp1-FX, served as an additional control and showed that sequences within the cotransfected plasmid were not responsible for the enhanced expression from pβCAT (Fig. 7). Similar results (data not shown) were obtained with pRSVCAT (Gorman et al. 1982b); the Rous sarcoma virus (RSV) promoter in this plasmid also does not contain a GC box.

These data showed that Sp1 is a limiting factor in CV-1 and COS-1 cells and suggested that the effect of stimulating its production could have profound effects on transcription of many genes.

Discussion

Cells infected with SV40 undergo profound changes, including alteration of the normal cellular controls. The primary viral factor responsible is T antigen. This oncogene product is known to interact with several regulatory proteins, including the cellular transformation-related protein p53 (Montenarh et al. 1986) and the product of the *Rb* susceptibility gene (DeCaprio et al.

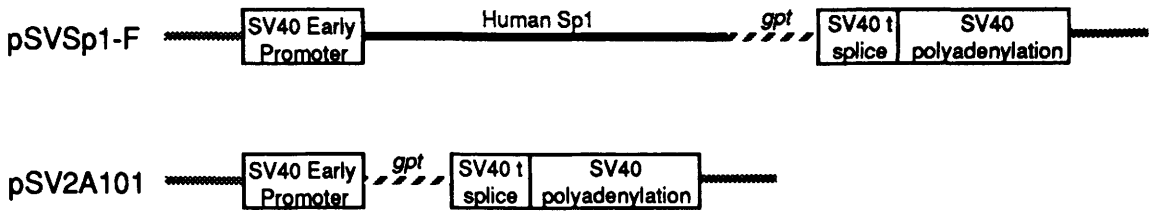

Figure 4. Vector for overexpressing human Sp1 in mammalian cells. The expression vector pSVSp1-F, is a derivative of pSV2Agpt (Kadesch and Berg 1986), in which the 5′ end of the *Escherichia coli* xanthine/guanine phosphoribosyltransferase (*gpt*) gene has been replaced by the Sp1 cDNA (Kadonaga et al., 1987). The regulatory signals are shown as boxes. A segment of the *gpt* gene (dashed line) has been retained to facilitate probing for the vector and its product in mammalian cells. Vector sequences from pBR322 are represented by shaded lines flanking the regulatory signals. The control vector pSV2A101 is identical, except that the Sp1-coding sequence is missing.

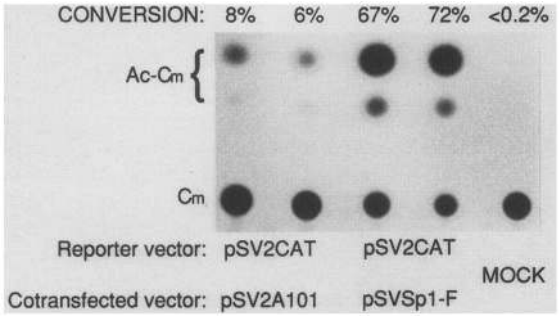

Figure 5. Overexpression of Sp1 activates the SV40 promoter at high copy number. COS-1 cells (10^6/75 cm^2) were transfected with 15 μg pSVSp1-F or pSV2A101 and 7.5 μg pSV2CAT with calcium phosphate (Saffer and Hughes 1986). CAT activity was determined (Gorman et al. 1982a) and is expressed above as percent of the total [^{14}C]-chloramphenicol converted to acetylated forms.

1988; Ludlow et al. 1989). For transcription factor AP-2 (Mitchell et al. 1987), direct interaction with T antigen alters functional activity. The data presented here show that an early viral gene product, most likely T antigen, altered the activity of the *trans*-acting factor, Sp1; but, in contrast to the above examples, it did so at the mRNA level.

When CV-1 cells were infected with SV40, the amount of Sp1 mRNA rose by approximately one order of magnitude. The RNA level increased beginning at 12 hr postinfection and reached a plateau at ~20 hr postinfection, remaining constant for at least another 16 hr. In our hands, the change in Sp1 was at least 2-fold greater than the stimulation of thymidine kinase mRNA, although thymidine kinase showed a 10- to 20-fold increase in other reports (Stuart et al. 1985; Stewart et al. 1987). The induction of Sp1 by viral infection was not simply due to stimulation of cell growth, because equal change was observed in logarithmically growing cells. Furthermore, it is important to note that AP-1 and CTF-1 message levels are unchanged for at least 24 hr after infection (J. Saffer, S. Jackson, and S. Thurston, unpubl.), indicating that SV40 activation of *trans*-acting factors at the mRNA level is not a general phenomenon.

The experiments presented here do not define the mechanism for the SV40 induction of Sp1 mRNA, but the altered expression of Sp1 was attributable to early gene activity. In this regard, it is of particular interest that T antigen may be an RNA-binding protein (Khandjian et al. 1982; Carroll et al. 1988). Another papovavirus protein, the 72-kD product of the adenovirus E2A gene, has been shown to contribute to the post-transcriptional regulation of mRNA (Cleghon and Klessig 1986; Lazardis et al. 1988). It is noteworthy that <3 kb of the 8.2-kb Sp1 message corresponds to protein-coding sequences. Perhaps the lengthy untranslated sequences play an important role in determining the regulation of Sp1 message stability. Alternatively, the SV40 early proteins may stimulate transcription of the Sp1 gene or improve the efficiency of Sp1 mRNA processing. Experiments are in progress to distinguish between these possibilities.

Although the mechanism for this specific mRNA induction remains to be determined, immunofluorescence has shown that the shift in Sp1 message is reflected at the protein level. There are several lines of evidence suggesting that an increased amount of Sp1 protein would have profound effects on cellular transcriptional activity and aid in viral transcription. In vitro assays using the SV40 promoter (Dynan and Tjian 1983a) and a monkey promoter (Dynan et al. 1985) have shown that transcription rates increase with higher amounts of Sp1. A similar dependence is seen in assays in vivo (Courey and Tjian 1988). Additionally, the affinity of Sp1 for different binding sites varies at least 10- to 20-fold (Briggs et al. 1986; Jones et al. 1986). Therefore, greater amounts of Sp1 will allow more sites to be populated.

The data presented show that an early event in the SV40-induced cellular alterations is stimulation of Sp1. According to the the above arguments, the higher level of Sp1 would directly benefit the virus in two ways. First, as the viral genome is replicated, the increased copy number of the viral regulatory region would require more of the factors such as Sp1 that are needed for viral gene transcription. Second, a greater abundance of Sp1 will increase expression of several cellular genes (e.g., those involved in nucleic acid metabolism like adenosine deaminase, dihydrofolate reductase, and hypoxanthine phosphoribosyltransferase) needed to support the viral life cycle.

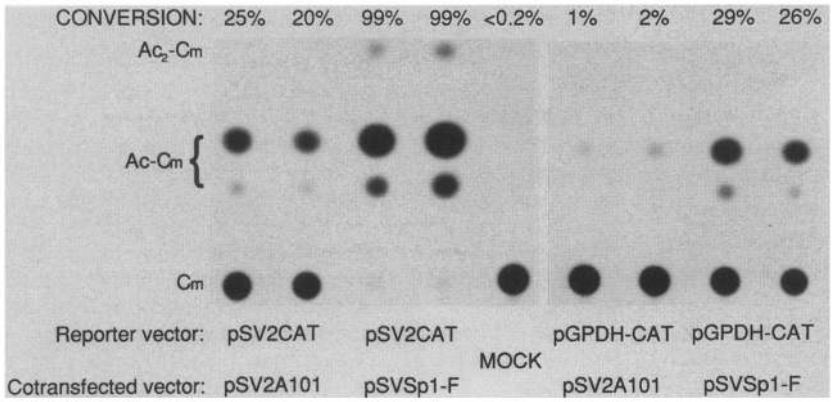

Figure 6. Overexpression of Sp1 activates Sp1-responsive promoters in CV-1 cells. CV-1 cells were transfected with pSV2CAT or pGPDH-CAT and either pSVSp1-F or pSV2A101 in an experiment analogous to that in Fig. 5. Note that the assay with pSV2CAT with pSVSp1-1 has progressed beyond the linear range; other assays (not shown) demonstrated that the SV40 promoter was activated 8- to 10-fold by overexpression of Sp1.

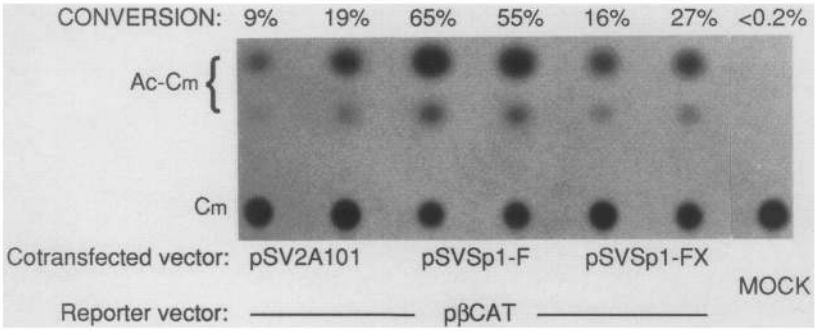

Figure 7. Overexpression of Sp1 activates the human β-globin promoter. CV-1 cells were transfected with pβCAT and either pSV2A101, pSVSp1-F, or pSVSp1-FX in an experiment analogous to that in Fig. 5.

To demonstrate directly that the heightened expression of Sp1 has a functional effect, we altered the expression of Sp1 in cells in the absence of other changes. For those experiments, we used a vector to overexpress Sp1 in monkey cells. In both CV-1 and COS-1 cells, we found CAT activity from pSV2CAT to be greatly stimulated by overexpression of Sp1. Thus, our results indicate that under these conditions, Sp1 levels are limiting and that the Sp1 induction will facilitate viral gene transcription. This contribution to the viral life cycle may be just one facet of the 10-fold increase in the level of Sp1. Alterations in the amount of Sp1 are also likely to affect the level of cellular gene transcriptional activity. Because the SV40 promoter has six clustered Sp1-binding sites and is very sensitive to Sp1, we also tested a typical cellular promoter. The GPDH promoter has a single GC box in addition to typical TATA and CCAAT boxes. Expression from this promoter was also stimulated by overexpression of Sp1.

Although the human β-globin and RSV promoters do not contain GC boxes, overexpression of Sp1 did enhance transcription from these promoters. There are several regions either within these promoters or in nearby vector sequences that have high similarity to the consensus GC box (Kadonaga et al. 1986). We estimate that Sp1 levels in the transfected cells may be as much as 100-fold greater than normal (data not presented); thus, the activation of these promoters may be due to Sp1 binding to the degenerate GC boxes, which act as fortuitous weak binding sites. Alternatively, enhanced β-globin and RSV transcription may occur through other mechanisms. For example, increased Sp1 levels may activate other transcription proteins which, in turn, make transcription more efficient from some genes. It is also possible that at high enough levels of Sp1, the effector domain interacts with the transcription complex without the otherwise required DNA binding. This possibility is supported by recent data showing synergistic activation of transcription by the glutamine-rich domains of Sp1 (Courey et al. 1989). However, in preliminary experiments using cotransfection assays, we did not see activation of transcription by overexpression of a truncated Sp1 missing the DNA-binding domain (J. Saffer, S. Jackson, and S. Thurston, unpubl.).

Although the data presented do not suggest any one mechanism for the increase in transcription of reporter genes when Sp1 is overexpressed, it is likely that several mechanisms contribute. It is clear, however, that increased expression of Sp1 can have profound effects on transcription, perhaps even on genes not normally regulated by Sp1. Such changes, as part of an overall viral strategy, may be an important contribution to T antigen-induced transformation.

Materials and methods

Cell cultures, viral infection, and transfection

CV-1 cells, a monkey kidney fibroblast line (Jensen et al. 1964), and COS-1 cells, an SV40-transformed derivative of CV-1 cells (Gluzman 1981), were maintained in Opti-MEM (GIBCO) supplemented with 2% fetal calf serum and penicillin/streptomycin. For viral infection, cells were routinely plated at a density of 1×10^6/75-cm² plate, and after 12–16 hr, were infected with SV40 (strain 776) at an MOI of 5.

For transfections, cells were plated at a density of 1×10^6/75-cm² plate. pSV40 is the entire SV40 genome cloned into the *Bam*HI site of pBR322 (oriented with the early region closest to the *Eco*RI site in pBR322). pSVE was derived from pSV40 by digesting pSV40 with *Nae*I and *Acc*I, followed by recircularization; this results in a vector containing SV40 sequences from nucleotide 345 through the viral origin to nucleotide 2533. Transfection with 22.5 μg of either plasmid used $CaPO_4$ as described previously (Saffer and Hughes 1986), except that the glycerol shock was omitted and the cells were washed after 24 hr.

Cotransfections for expression assays were similar with 7.5 μg reporter vector and 15 μg either pSVSp1-F or pSV2A101. pSV2A101 was constructed by replacing a *Hin*dIII–*Kpn*I restriction fragment at the amino terminus of the *gpt* gene in pSV2Agpt (Kadesch and Berg 1986) with the oligonucleotide 5′-AGCTATGAATTCAGATCTAAGCTTGTAC-3′, 3′-TACTTAAGTCTAGATTCGAA-5′. This adapter provides convenient *Eco*RI, *Bgl*II, and *Hin*dIII restriction sites for the insertion of the Sp1 cDNA and an in-frame AUG codon. pSVSp1-F was constructed by inserting an *Eco*RI–*Hin*dIII fragment from pSp1-1 (Kadonaga et al. 1987) into pSV2A101. pSVSp1-FX was constructed by cutting pSVSp1-F at the *Eco*RI site at the 5′ end of the Sp1 gene, filling in the overhanging ends with Klenow, and ligating in an 8-bp *Xho*I linker (5′-CCTCGAGG). A reporter vector containing the human β-globin promoter driving CAT expression was created by replacing the *gpt* gene of pβ.LR (Saffer and Thurston 1989) with the CAT gene. The reporter vector pGPDH-CAT was a gift from Dr. L. Kozak and the vectors pSV2CAT and pRSVCAT were obtained from the American Type Culture Collection (Rockville, Maryland). Protein was isolated from transfected cells after 48 hr by repeated freeze–thaw cycles in 100 μl 0.25 M Tris (pH 7.8). Enzyme

assays were performed in duplicate, as described by Gorman et al. (1982a), and the radioactivity in each spot was quantitated by liquid scintillation counting or with an AMBIS Radioanalytic Imaging System (San Diego).

RNA isolation and analysis

Total RNA was isolated by the guanidine hydrochloride (GuHCl) method, as described (Jacobsson et al. 1985), except that the GuHCl was added directly to cells that had been washed twice with phosphate buffered saline (PBS). Cell lysates were then passed through 25-gauge needles ~10 times to shear the DNA. RNA samples (5 μg) were electrophoresed through 1.2% agarose/2.2 M formaldehyde gels (Maniatis et al. 1982) and transferred to a nylon membrane (Zeta-bind, AMF-CUNO). Blots were then UV-shadowed and photographed (Thurston and Saffer 1989) to confirm equal loading and transfer. Hybridization conditions were a modification of Church and Gilbert (1984). Blots were hybridized in 7% SDS/0.5 M sodium phosphate/2 mM EDTA/0.1% sodium pyrophosphate at 65°C for 16–40 hr with 10 μg/ml poly(A) and 50 μg/ml sheared salmon DNA as competitors. DNA probes were uniformly labeled with [α-^{32}P]dCTP to a sp. act. of 2×10^8 to 2×10^9 dpm/mg by the random primer method. The Sp1 probe is a 2.8-kb *Eco*RI fragment from the human cDNA clone pSp1-1 (Kadonaga et al. 1987; a gift from R. Tjian). The human thymidine kinase and β-2 microglobulin cDNA clones were a gift from S. Conrad (Stuart et al. 1985). Quantitation of autoradiographs was performed with a Bio-Rad model 620 Densitometer.

Immunofluorescence

CV-1 cells were scraped into PBS, diluted to a density of 2×10^5 cells/ml, and cytocentrifuged onto gelatin-coated slides. After air-drying for 1 hr, the slides were fixed in acetone for 20 min at −20°C, air-dried again, and stored frozen until used. Cells were hydrated for 15 min in Dulbecco's modified Eagle medium with 10% fetal calf serum and then reacted for 30 min at room temperature with either a rabbit polyclonal anti-Sp1 antibody or the mouse monoclonal anti-T antigen antibody pAb419 (Harlow et al. 1981) in the same medium. Cells were washed extensively with PBS and reacted as above with either fluorescein-conjugated goat anti-rabbit (for the anti-Sp1) or rhodamine-conjugated goat anti-mouse (for pAB419). Slides were washed again with PBS and mounted for examination. For double labeling, the second antibodies were fluorescein-conjugated goat anti-mouse IgG and rhodamine-conjugated goat anti-rabbit IgG (Cappel, Malvern).

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