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Control of Simian Virus 40 Gene Expression at the Levels of RNA Synthesis and Processing: Thermally Induced Changes in the Ratio of the Simian Virus 40 Early mRNA's and Proteins

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Examination of the simian virus 40 early mRNA's from infected AGMK or CV-1 cells showed that the ratio of large T- to small t-antigen mRNA's increased with an increased incubation temperature. In *tsA*58 mutant-infected cells, an increased incubation temperature resulted in the overproduction of early RNAs; however, the ratio of the early mRNA's was the same, at any temperature, in both wild-type- and *tsA*58-infected cells. Thus, the thermally induced alteration in the early mRNA ratios was apparently not affected by the *tsA* mutation or by the overproduction of early RNA in *tsA* mutant-infected cells. Time course studies at various temperatures showed that, although the ratio of large T- to small t-antigen mRNA's increased with temperature, at any one temperature it was constant from early to late times of infection. Furthermore, the ratio of the early mRNA's adjusted in temperature shift experiments. Thus, the ratio of the early mRNA's appeared to be intrinsic to the thermodynamic environment of the cell. The thermally induced alterations in the early mRNA's were reflected at the protein level by parallel changes in the ratio of large T- to small t-antigens. These data suggest a level of gene expression control which may function at the stage of splicing.

The small DNA tumor viruses have provided excellent model systems for studying the control of gene expression. In this regard, simian virus 40 (SV40; see Fig. 1) has been particularly valuable for the analysis of transcriptional and post-transcriptional regulatory mechanisms. The temporal expression of early and late SV40 genes appears to be controlled at the level of transcription (2, 5, 15, 21). These studies showed that a primary factor in determining the transcriptional activity of the SV40 genes is one of the early gene products, large T-antigen, which is thought to have a repressor-like activity in modulating early transcription. These conclusions are based, in part, on studies with early SV40 temperature-sensitive mutants (*tsA* mutants). At elevated temperatures these mutants become defective in large T-antigen function (1a, 19, 28, 30) and overproduce early viral RNA (2, 15, 21).

The primary early and late transcripts are modified subsequent to their synthesis by capping, polyadenylation, internal methylation, and splicing. All of these modifications are also found in the biosynthesis of eucaryotic mRNA's. What appears, thus far, to be unique for viral mRNA's is the observation that one primary transcript may be the precursor for different mRNA's through alternate splicing events. For example, a single primary transcript of the SV40 early coding region may be the precursor for the mRNA coding for either small t- or large T-antigen, depending on the location of the splicing event (4, 6). If this is the case, then the selection of the splicing event provides a potential regulatory step in early SV40 gene expression, and factors influencing the relative frequency of either splice will control the relative abundance of the two early SV40 proteins, large T- and small t-antigens.

The study reported here was initially undertaken to determine whether large T-antigen regulates not only the rate of synthesis of primary early transcripts, but also the relative amounts of large T- and small t-antigen mRNA's. Our results indicated a much more interesting phenomenon; we found in both wild-type (WT)- and *tsA* mutant-infected cells that the ratio of large T- to small t-antigen mRNA's increased and decreased with incubation temperature. These thermally induced alterations in the early mRNA ratios were reflected at the protein level by similar alterations in the large T- to small t-

antigen ratio. Thus, the thermal (or the general thermodynamic) environment of the cell alters the expression of the early genes, indicating a level of gene expression control which may function at the level of splicing.

MATERIALS AND METHODS

Cells and viruses. Primary African green monkey kidney cells (AGMK) and the established AGMK line CV-1 were cultivated in minimal essential medium plus 10% fetal calf serum. The virus strains used were the WT strain VA4554 (25) and a temperature-sensitive mutant derived from it, *tsA*58 (26, 27).

Infection of cells and preparation of cytoplasmic RNA. Confluent monolayers of cells in 150-cm^2 Costar plastic bottles were infected at a multiplicity of 10 PFU/cell in 5 ml of minimal essential medium containing 2% fetal calf serum. After rocking at room temperature for 1.5 h, 20 ml of medium was added, and the cells were incubated at 32°C for 40 h. At this point, cells were harvested (32°C samples) or shifted to 41°C for 5 h (shifted samples) before harvesting. Other cultures were infected for 24 h at 41°C before harvesting (41°C samples). Cytoplasmic RNA from infected cells was prepared as previously described (13). Polyadenylated RNA was selected on columns of oligodeoxythymidylic acid-cellulose (3).

Preparation of ^{32}P-labeled SV40 DNA hybridization probes. SV40 DNA was labeled in vivo and purified as previously described (16). The labeled form I SV40 DNA was digested with appropriate restriction endonucleases (New England BioLabs, Waltham, Mass.), using conditions indicated by the company. The reaction mixtures were phenol extracted and ethanol precipitated, and the strands of the fragments were separated by the method of Hayward (10). Separated strands were electroeluted, self-annealed, and chromatographed on hydroxyapatite. The resulting purified single strands were dialyzed into 10 mM Tris-hydrochloride (pH 7.5)–1 mM EDTA.

Hybridization of single-stranded DNA probes and cytoplasmic RNA. To maintain conditions of DNA excess, the amount of RNA used in each hybridization was varied according to the abundance in the sample. DNA excess conditions were established by titration of different samples with separated strand probes containing the early coding region. Samples contained 0.5 to 10 μg of polyadenylated RNA or 5 to 70 μg of nonpolyadenylated RNA. For each reaction, 10 ng (10,000 cpm) of DNA probe was added. The RNA and probe were ethanol precipitated together in silane-treated tubes. The pellets were dried in a vacuum and then dissolved in 30 μl of 50% formamide–0.4 M NaCl–0.04 M PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)] (pH 6.5). The samples were hybridized at 37°C for 24 to 72 h. Long hybridization times were used to insure that the reactions were complete; this varied with the sample and had been previously determined.

Analysis of hybrids by nuclease S1 digestion and alkaline gel electrophoresis. After hybridization, the samples were treated with nuclease S1 and electrophoresed on 1.4 or 1.8% alkaline agarose gels as described by Berk and Sharp (4). The gels were dried on DEAE paper and autoradiographed at −70°C, using Kodak XR-1 X-ray film and a Du Pont Cronex Lightning-plus intensifying screen.

Analysis of [^{35}S]methionine-labeled viral proteins. For the analysis of the ratio of large T- to small t-antigens, confluent monolayers of AGMK or CV-1 cells, grown in 50-mm plates (three plates per set), were infected with VA4554 or *tsA*58 at a multiplicity of 10. The infected cells were incubated for 40 h at 32°C. At this point, the cells were pulse-labeled for 1 h at 32°C with [^{35}S]methionine or shifted to 41°C for 4 h before pulse-labeling for 1 h at 41°C. A third set of cultures was infected entirely at 41°C for 24 h and then pulse-labeled for 1 h. The pulse-labeling was accomplished by washing the cells three times with appropriately warmed (32 or 41°C) methionine-free minimal essential medium containing 2% dialyzed fetal bovine serum and then labeling each plate with 1 ml of prewarmed medium containing 200 μCi of [^{35}S]methionine (300 Ci/mmol; Amersham Corp., Arlington Heights, Ill.). After labeling, the plates were put on ice and washed three times with cold analysis buffer (Tris-buffered saline, pH 8, containing 1 mM dithiothreitol, 300 μg of phenylmethylsulfonyl fluoride per ml, and 150 μg of tosylamid-2-phenylethylchloromethyl ketone per ml). The cells were scraped into 1.5 ml of analysis buffer, sedimented at 3,000 rpm for 10 min, suspended in analysis buffer containing 0.5% Nonidet P-40, incubated on ice for 30 min, and vigorously blended in a Vortex mixer every 5 min. The lysates were sedimented at 15,000 rpm for 60 min, and the supernatant fraction was withdrawn and saved. Samples (0.3 ml) of the supernatants were immunoprecipitated with heat-inactivated *Staphylococcus aureus* and antiserum (directed against the SV40 early proteins) as described by Jay et al. (12). The serum had been titrated previously to determine optimal conditions for these samples. Both the serum and *S. aureus* were the gift of Gilbert Jay. Immunoprecipitates (in 15- to 20-μl volumes) were analyzed by electrophoresis on 10% sodium dodecyl sulfate-polyacrylamide slab gels (11). Radiolabeled proteins were detected by fluorography (17). After location of specific bands on the gels, the dried gel segments containing them were excised and rehydrated in water for 20 min at room temperature. The gel segments were then equilibrated in dimethyl sulfoxide to remove the diphenyloxazole used in fluorography (five washes, 2 ml of dimethyl sulfoxide each, for 20 min at room temperature). The gel slices were equilibrated in water and then treated with 1 ml of 0.8% ammonium bicarbonate containing 60 μg of trypsin for 12 h at 37°C. The mixture was then counted in 10 ml of Aquasol (New England Nuclear Corp., Boston, Mass.).

RESULTS

Experimental approach. Total cytoplasmic or polyadenylated cytoplasmic RNA (both of which gave identical results in the following experiments) was isolated from SV40-infected monkey kidney cells under a variety of experimental conditions. These RNAs were annealed with ^{32}P-labeled, separated, single-stranded DNA probes containing the entire known early coding regions of the SV40 genome. The hybrid

molecules were assayed by the S1 nuclease technique of Berk and Sharp (4), followed by alkaline agarose gel electrophoresis and autoradiography. For the quantitation of the amounts of mRNA species from autoradiographic band intensities, hybridization reactions were performed in DNA excess, and incubation times were prolonged to insure that reactions had gone to completion.

Synthesis of large T- and small t-antigen mRNA's. Previous experiments showed that early SV40 RNA is overproduced in the absence of a functional large T-antigen (2, 15, 21). We wanted to determine whether the large T-antigen was involved (i) only in the control of initiation of any RNA synthesis from the early coding region, presumably by operating on the promoter, or (ii) in regulation of the amount of large T-antigen mRNA relative to small t-antigen mRNA. Parallel cultures of AGMK cells were infected with an early SV40 temperature-sensitive mutant (*tsA*58) at the permissive temperature (32°C) for 40 h. At that time, one culture was held at 32°C (32°C samples), and the other was shifted to the nonpermissive temperature (41°C) for 5 h (shifted samples). In a control experiment, similar RNA samples were prepared from parallel cultures infected with WT SV40. Samples of the RNAs purified from these cultures were annealed with a ^{32}P-labeled SV40 DNA probe representing the early strand of an *Hpa*II-*Bam*HI fragment (0.14 to 0.72 SV40 map unit [m.u.] [Fig. 1]) which contains the early coding region (input amounts of RNA varied due to overproduction in the *tsA*-shifted samples; see legend to Fig. 2). The analysis of the RNAs from these cultures is shown in Fig. 2. In each track, there are three prominent bands (2,000, 580, and 320 nucleotides [n]) which represent the common early RNA body (2,000 n) and the leaders for small t-antigen mRNA (580 n) and large T-antigen mRNA (320 n) (4; Fig. 1). In addition, a 1,900-n band was often seen, which arises due to the 3′ overlap of early and late SV40 mRNA's which causes probe displacement (1). Under DNA probe excess hybridization conditions, the relative band intensities of the 580- and 320-n segments corresponded to

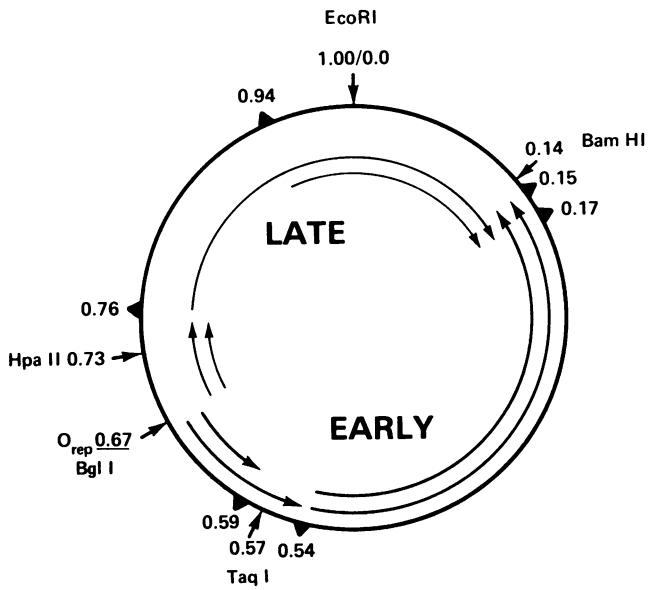

FIG. 1. *Map of the SV40 genome. The heavy outer circle represents the viral DNA with the restriction endonuclease cleavage sites indicated by arrows. O_{rep} denotes the origin of DNA replication, the site at which large T-antigen binds (20, 31) and the presumed site of its regulatory control on early RNA synthesis (2, 15, 21). The triangles mark the positions of RNA endpoints (0.15 and 0.17 m.u.) and splice junctions (0.59, 0.54, 0.76, and 0.94 m.u.). The counterclockwise arrows indicate the map locations of the two major polyadenylated cytoplasmic mRNA's found early in viral infection, i.e., before the onset of viral DNA synthesis. The early RNA coding for large T-antigen has a leader which extends from 0.660 to 0.600 m.u., spliced to a "body" extending from 0.533 to 0.154 m.u. The other early RNA, coding for small t-antigen, consists of a larger leader (0.660 to 0.546 m.u.), spliced to the same body as the large T-antigen mRNA. The clockwise arrows indicate the map locations of the additional polyadenylated cytoplasmic mRNA's found late in infection (i.e., after the onset of viral DNA replication), the 19S and 16S RNAs, which code for the viral structural proteins. Map positions are derived from references 4, 7, 9, 16, 18, and 19.*

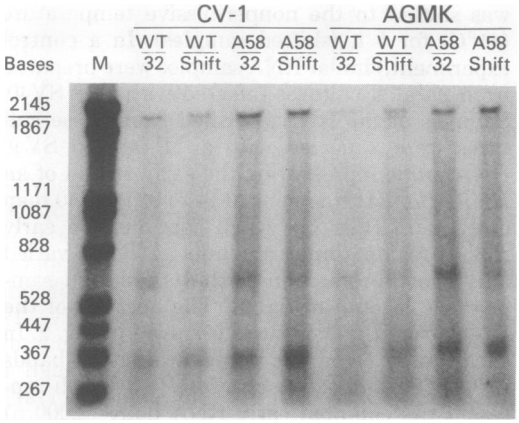

FIG. 2. *Nuclease S1 analysis of SV40 cytoplasmic early mRNA. Total cytoplasmic RNA isolated from CV-1 or AGMK cells infected with WT VA4554 (WT) or tsA58 virus was hybridized to the early (minus) strand of the larger fragment of ^{32}P-labeled SV40 DNA generated by digestion with the restriction endonucleases HpaII and BamHI (0.14 to 0.73 m.u. [Fig. 1]). Samples were hybridized, nuclease S1 treated, electrophoresed, and autoradiographed as described in the text. The 32°C samples (32) were harvested after 40 h of infection at 32°C. The shifted samples (shift) were harvested after a 5-h shift from 32 to 41°C. The input amounts of RNA varied among samples to maintain SV40 DNA excess hybridization conditions. Input RNA amounts were as follows: 64 and 54 μg, respectively, for WT-infected CV-1 cells at 32°C and shift; 22 and 5 μg, respectively, for tsA58-infected CV-1 cells at 32°C and shift; 14 and 9 μg, respectively, for WT-infected AGMK cells at 32°C and shift; and 7 and 2.5 μg for tsA58-infected AGMK cells at 32°C and shift. Lane M contains SV40 DNA fragment markers. Similar results were obtained with polyadenylated cytoplasmic RNAs. It will be noted that in some of the samples, the 2,000-n band is not as intense as would be expected from comparison with the 580- and 300-n bands. This occurs in some samples due to the long hybridization times necessary to obtain complete hybridization reactions. This causes some breakdown of the RNA, having the greatest effect on the detection of larger segments. This did not occur in most of the samples used to determine the ratio of large T- to small t-antigen mRNA's. The data above were chosen for reproduction in this paper because they clearly show all of the results discussed on one gel.*

the relative amounts of the small t- and large T-antigen mRNA's, respectively. Inspection of the relative intensities of these bands in Fig. 2 suggested that after a shift to 41°C, the amount of large T-antigen mRNA relative to small t-antigen mRNA increased significantly, both in cells infected by *tsA58* and in cells infected by WT SV40. A similar shift toward production of large T-antigen mRNA at the higher temperature was seen in CV-1 cells infected either by WT SV40 or by *tsA58* (Fig. 2). For the quantitation of the relative amounts of the early SV40 mRNA's, autoradiograms similar to those shown in Fig. 2 were analyzed by microdensitometry (Table 1). Although there was two to three times as much large T-antigen mRNA as small t-antigen mRNA at 32°C, this ratio increased to between five and seven times during shift-up conditions for both WT- and *tsA58*-infected cultures. When infected AGMK cells were incubated continuously at 41°C (41°C samples), the ratio of large T- to small t-antigen mRNA's was even greater (8- to 11-fold).

The experiments above show that there was a substantial increase in the ratio of large T- to small t-antigen mRNA's with an increase in the temperature of incubation. It can be argued that, during the course of a lytic infection at any temperature, the ratio of the early mRNA's may change with time. Thus, in the shift from 32 to 41°C the lytic process may have been accelerated to a later phase with a higher ratio of large T- to small t-antigen mRNA's. Likewise, the 24-h infection at 41°C may represent an even later phase of infection. To determine whether this was the source of the ratio changes, AGMK cells were infected with WT SV40 for various times at 32, 37, and 41°C; the ratios of cytoplasmic early mRNA's were then determined as described above. Table 2 shows that the specific early mRNA ratio for any of the temperatures remained the same from early to late times of infection at that temperature. In addition, the ratio of early mRNA's adjusted to the expected values in temperature shift-up and shift-down experiments (data not shown). Thus, the ratio of large T- to small t-antigen mRNA's appeared to be an intrinsic property determined by the thermal environment of the infected cell. Since the temperature shift experiments (Table 1)

TABLE 1. *Ratio of large T- to small t-antigen mRNA's in WT- and tsA58-infected cells under various temperature conditions*[a]

Infecting virus	AGMK cells			CV-1 cells		
	32°C[b]	Shift[c]	41°C[d]	32°C[b]	Shift[c]	41°C[d]
VA4554 (WT)	2.1	5.7	8.8	2.4	5.2	ND[e]
tsA58	2.7	6.6	10.7	2.8	6.3	ND

[a] Ratios were determined by microdensitometer tracing of the 580- and 320-n bands of autoradiographs such as those shown in Fig. 2. The values shown are the average of at least two determinations and are multiplied by 1.72 to correct for the difference in sizes of the leader segments for large T- and small t-antigen mRNA's determined from nucleotide sequence data (18, 19). All numbers have a standard error of ±0.20.

[b] Infection at 32°C for 40 h.

[c] Infection at 32°C for 40 h with a 5-h shift to 41°C.

[d] Infection done entirely at 41°C for 24 h.

[e] ND, Not determined.

showed that the early mRNA ratios varied with temperature identically in WT- and *tsA*58-infected cells, we also conclude that large T-antigen (at least the portion affected by the *tsA*58 mutation) and overproduction of early mRNA have no significant effect on the early mRNA ratio.

Effect of thermally induced alterations of early mRNA ratios on the synthesis of large T- and small t-antigens. We next wanted to determine whether the temperature-dependent alterations in the early mRNA ratio were reflected at the protein level. To determine this, AGMK or CV-1 cells were infected with WT or *tsA*58 at (i) 32°C for 40 h; (ii) 32°C for 40 h, followed by a 5-h shift to 41°C; and (iii) 41°C for 24 h. During the last hour of each infection period, the cells were pulse-labeled with [^{35}S]-methionine. After extraction and immunoprecipitation with anti-T-serum, the early proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 3). As expected from a previous study (26, 29), *tsA*58-infected cells synthesized more large T-antigen after a shift to 41°C. Small t-antigen synthesis was also increased in these cells. In the exposure of the gel shown in Fig. 3, the small t-antigen bands were not visible in the WT-infected CV-1 cell samples; however, they were easily detected upon longer exposures (data not shown). For a determination of the ratio of large T- to small t-antigens in these samples, the bands containing the pulse-labeled polypeptides were excised from the gel and counted as described above. Table 3 shows the ratio of large T- to small t-antigens determined from the counts in the gel bands; these ratios have been corrected for the difference in the number of methionines in the two proteins as determined from the nucleotide sequence (9, 19). The ratios of the early proteins at the various incubation temperatures varied in a fashion similar to the ratio of their mRNA's.

TABLE 2. *Ratios of large T- to small t-antigen mRNA's in WT-infected AGMK cells from early to late times of infection at 32, 37, and 41°C*[a]

32°C		37°C		41°C	
Time postinfection (h)	Ratio	Time postinfection (h)	Ratio	Time postinfection (h)	Ratio
12	2.1	8	4.3	6	8.8
24	2.4	21	4.4	12	8.5
48	2.3	40	4.2	24	8.1

[a] Ratios were determined as described in Table 1, footnote *a*, and the text. AGMK cells were infected with WT VA4554 at 32, 37, and 41°C at 10 PFU/cell. At the noted times postinfection, the total cytoplasmic RNA was harvested and analyzed.

Thus, the results show that the thermal effects on the early mRNA ratios were reflected at the protein level.

DISCUSSION

The absolute amounts of SV40 early mRNA's in WT- and *tsA*58 mutant-infected cells at different temperatures are not the same (2, 15, 21); however, the relative ratio of large T- to small t-antigen mRNA's varied similarly with temperature in both WT- and *tsA*58 mutant-infected cells (Table 1). Thus, it appears that large T-antigen does not influence the determination of the ratio of the two early mRNA's. Although it is conceivable that the *tsA* mutant large T-antigen may be temperature insensitive for such a regulatory function, this appears unlikely. For example, Tegtmeyer et al. (29) have shown that the *tsA* large T-antigen is rapidly degraded at the nonpermissive temperature. It seems improbable that a fragment of the mutant protein could maintain WT activity for this function. In addition, Handa and Sharp (personal communication) have shown that the ratio of the two early SV40 mRNA's at 37°C is not altered by the presence of inhibitors such as emitine and cycloheximide which block the synthesis of the early proteins. We conclude, therefore, that large T-antigen controls early gene expression primarily by general regulation of all early RNA transcription, presumably by functioning on the promoter. The relative proportions of large T- and small t-antigen mRNA's appear to be determined by other factors which are unaffected by the *tsA* mutation or the overproduction of early RNA in *tsA* mutant infections.

The thermally induced variations in the early mRNA (and protein) ratios may occur at several levels. Recent sequencing studies (18) have shown two different 5′ termini for the early SV40 mRNA's (within 7 n of each other). Although no evidence suggests that either terminus is specific for one of the early mRNA's, these data may indicate that there are two early promoters. Thus, thermal factors may alter the early mRNA ratio by influencing promoter selection.

Another possible mechanism for altering the ratio of the early mRNA's derives from the fact that both early mRNA's share the same genomic sequences, differing only in their splice positions. Thus, the thermally induced alterations in the mRNA ratios may involve the relatively small sequence differences which determine large T- and small t-antigen mRNA's (in the 0.534 to 0.60-m.u. region [Fig. 1]). For example, thermal effects could alter the activity of a nuclease(s), thus differentially affecting the early mRNA stabilities, or alter the transport mechanism such that the rate of transport of specific mRNA

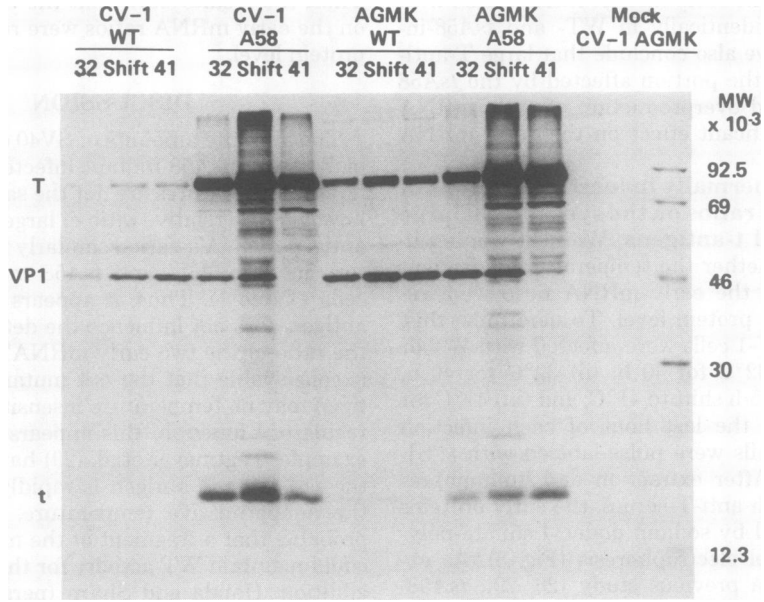

FIG. 3. *Examination of pulse-labeled SV40 early proteins. Cultures of CV-1 or AGMK cells were infected with WT VA4554 (WT) or tsA58 virus. The infection conditions were as follows: (i) 32°C for 40 h (32); (ii) 32°C for 40 h, followed by a 5-h shift to 41°C (shift); (iii) 41°C for 24 h (41). During the last hour of infection at the various temperatures, the proteins were pulse-labeled with [^{35}S]methionine as described in the text. The labeled proteins were extracted, immunoprecipitated and analyzed on a sodium dodecyl sulfate-polyacrylamide gel (see text) with appropriate markers. The positions of large T-antigen (T) and small t-antigen (t) are indicated. In addition, the serum used in this experiment precipitated the viral coat proteins (predominantly VP1). Note that the 41°C samples from cells infected with tsA58 contain little or no VP1, as expected for a tsA mutant infection.*

TABLE 3. *Ratios of large T- to small t-antigen proteins in WT- and tsA58-infected cells at various incubation temperatures*[a]

Infecting virus	AGMK cells			CV-1 cells		
	32°C	Shift	41°C	32°C	Shift	41°C
VA4554 (WT)	2.5	4.6	5.5	1.8	3.5	4.9
tsA58	2.1	8.6	10.6	1.8	4.7	6.0

[a] Ratios were determined from counts in pulse-labeled immunoprecipitated large T- and small t-antigens after separation on a sodium dodecyl sulfate-polyacrylamide gel (Fig. 3). The ratios are corrected for differences in numbers of methionines between the two proteins as determined from the nucleotide sequence (9, 19).

species changes. It seems likely that the two early mRNA's are derived from a common primary transcript of the early coding region by different splicing events. With this consideration, the thermal effects on the early mRNA ratios may result from an alteration of sequence recognition by a splicing enzyme(s) such that the relative frequency of splicing events changes or from an alteration of the stability of secondary structures in the primary RNA transcript which are required for the splicing events, thus changing the equilibrium of splice reactions.

These latter two possibilities are supported by previous studies (2, 21) which indicate that the total amount of early RNA (or precursor) is relatively equal in the cytoplasm and nuclei of WT- infected cells at 32°C and after a shift to 41°C. This suggests that the ratio changes occur by qualitative variation within a fixed amount of precursor. In addition, examination of deletion mutants in the 0.535- to 0.60-m.u. region, the unique coding region for small t-antigen (Fig. 1), has yielded information about possible sequence requirements for the splicing of early mRNA's (14; G. Khoury, J. C. Alwine, R. Dhar, P. Gruss, C.-J. Lai, S. Segal, and I. Seif, Cold Spring Harbor Symp. Quant. Biol., in press). None of these viable deletion mutants affects the ability to produce the large T-antigen mRNA splice. Since these mutants include deletions which remove the small t-antigen mRNA splice donor sequence at 0.546 m.u. (18, 24), it is unlikely that the small t-antigen mRNA splice is a prerequisite for large T-antigen mRNA synthesis. On the other hand, specific deletions within this region which do not involve suspected splice junctions will greatly decrease the amount of stable small

t-antigen mRNA produced. This indicates that specific sequences, well removed from the splice junctions, may be important for this splice (14; Khoury et al., in press). It has been suggested from these data that these deletions may alter conformations in primary transcripts which are required for the splice. If this is the case, it is possible that thermal (or thermodynamic) effects which alter the stabilities of these conformations change the rates of splicing events and, hence, the ratios of the early mRNA's. Another indication of the importance of sequence in splicing is noted in the observation that many RNA's are spliced identically in several different cells (Khoury et al., in press). Our examination of early mRNA's in SV40 (VA4554)-transformed hamster cells demonstrates that not only are the splices the same, but also the ratios of the early mRNA's vary with temperature in the same way as in lytically infected monkey cells (data not shown).

Although these data indicate that information within the sequence may be involved in the determination of the early mRNA ratio, we cannot rule out a high degree of conservation of splicing factors between species. In this regard, the requirement for factors other than primary RNA sequence is implied by the demonstration of complete unspliced early transcripts in SV40-infected mouse teratocarcinoma cells before differentiation; after the induction of cell differentiation by retinoic acid, correctly spliced early mRNA's are found (22, 23). This implies that factors may be induced during differentiation which directly or indirectly affect splicing of RNA molecules.

Thus, there are several possible levels at which the environment of the cell may influence the early mRNA ratios. Whatever the mechanism, these changes in early mRNA ratios indicate a potentially important level for control of gene expression. In a general model, this control may allow a DNA sequence which encodes information for more than one product to differentially express the products by altering the processing which produces the final mRNA's. This differential expression may be determined by temperature or other thermodynamic variables (pH and ionic strength) of the environment of the cell.

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