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Characterization of two SV40 early mRNAs and evidence for a nuclear "prespliced" RNA species

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ABSTRACT

Using in vitro translation of sucrose-gradient fractionated cytoplasmic mRNA from SV40-infected cells, we have shown that a deletion in the region mapping between 0.54-0.59 reduced the size of mRNA for small-t but not the size of mRNA for large-T. Mutants with a deletion in this region were shown to produce in vivo either shortened small-t or no small-t, and normal large-T. Similarly, in vitro translation of poly(A)+cytoplasmic RNA from cells infected with these mutants gave the same results. On the other hand in vitro translation of poly(A)+nuclear RNA from the mutants which made no small-t produced a small-t derivative possibly synthesized from a prespliced RNA species. We have also shown that poly(A)+nuclear RNA from mutant dl 2122 produced two small-t related proteins: one of these (MW: 11K) probably represents the product of a "prespliced" RNA, the other (MW: 17K)which is also found in the cytoplasm represents the product of the mutant specific small-t mRNA.

INTRODUCTION

Recently it has been found that SV40-infected or transformed cells produced two viral proteins both immunoprecipitable with anti-Tumor (T) serum, termed small-t (approximately 19 K daltons) and large-T antigens (approximately 90 K daltons) respectively (1, 2, 3, 4, 5). Both of these early proteins may be involved in the establishment or the maintenance of the transformed state (6, 7, 8, 9, 10). The two early proteins contain a number of common tryptic peptides (2, 11) and have identical amino-termini mapping at 0.65 map units (12).

Among the deletion mutants of SV40, one group (dl 54/59) of viable mutants contains various deletions between map coordinates 0.54 and 0.59 (13) and is of particular interest in the study of small-t and large-T. Crawford et al. (6) have examined the synthesis of these early proteins after infection of CV-1 (monkey) cells with wild type and deletion mutants of SV40, and have shown that the segment of SV40 DNA mapping between 0.59 and 0.55 is es-

sential for the production of full sized small-t but not of large-T, whereas the nucleotide sequence between 0.21 and 0.18 encodes structural information for large-T but not for small-t. New mutants of the dl 54/59 group have been isolated by Feunteun et al. (8) and Sleigh et al. (9). It has been shown that in vivo, the dl 54/59 mutants produce a shortened small-t, or fail to produce a detectable small-t whereas they produce normal sized large-T (6, 8, 9). Recently Berk and Sharp (14) have determined the structure of two early mRNAs of SV40 and have suggested that they correspond to the messengers for two viral antigens. Each of these two mRNAs are spliced, i.e. they are single covalently continuous RNA molecules arising from well-separated regions on the viral genome.

All the data presently available suggest the following model (6): (i) The mRNA for large-T would consist of sequences mapping from 0.65 to 0.60 and 0.54 to 0.17. Since the deleted sequences cover that region around 0.55 to 0.53 which contains multiple termination codons in all three reading frames (15, 16) the mRNA would be translated from the initiation codon (at 0.65) to a termination codon at 0.174 (16). (ii) The mRNA for small-t would consist of sequences mapping from 0.65 to 0.54 and from 0.54 to 0.17 and the small-t antigen would be translated from 0.65 to 0.55 where the termination codons are conserved.

The present work was undertaken in order to characterize the messengers for small-t and large-T and to gain further insight into the mechanism of the splicing which yields small-t mRNA.

MATERIAL AND METHODS

The dl 54/59 mutants 2102, 2112, 2122 (8) and their parental wild-type SV40 large plaque, SV-1 (LP) strain (17) were plaque purified, grown under low multiplicity conditions (0.005 PFU/cell), and titered on CV-1 (monkey kidney) cell cultures. The dl 54/59 mutants 883 and 891 (13) and their parental wild-type SV40 strain 776 were obtained from Dr. J. Feunteun. Dl 891 was plaque-purified in our laboratory. The SV40 mutant dl 1263 was obtained from Dr. L. Crawford (10).

Primary baby mouse kidney (BMK) cell cultures (18) were prepared and grown under conditions previously described (19) in 10-cm plastic petri dishes. The cultures were infected 4 days after seeding, at a multiplicity of about 50 PFU/cell. After a 1-hour adsorption at 37°C, cells were supplemented with Dulbecco's modified Eagle's medium without added serum.

Cell fractionation and isolation of poly(A)+cytoplasmic and nuclear RNA.

Twelve hours after infection, 40 cultures of SV40-infected mouse kidney cells were washed twice with 5 ml each of ice-cold isotonic buffer I (10 mM triethanolamine pH 8, 25 mM NaCl, 5 mM $MgCl_2$, 0.25 M sucrose); 0.5.ml per dish of cold buffer I containing 1 % Nonidet P-40 (NP-40, Shell Chemical Co) was then added for 5 minutes in order to lyse the cells. The homogenate was scraped into a glass centrifuge tube, and nuclear and cytoplasmic fractions were separated by centrifugation at 2500 rpm for 5 minutes at 4°C. Cytoplasmic RNA was extracted by the phenol-chloroform method (20) ; nuclear RNA was extracted by method of Scherrer (21) including a DNAse treatment. Cytoplasmic and nuclear RNAs were fractionated by oligo-(dT)-cellulose chromatography (20) in order to obtain the poly(A)+RNA. After ethanol-precipitation poly(A)+cytoplasmic (or nuclear) RNA was dissolved in 100 or 50 µl H_2O to give an RNA concentration of 500-1000 µg/ml.

Translation of poly(A)+cytoplasmic or nuclear RNA.

The poly(A)+RNA was translated in the micrococcal nuclease treated rabbit reticulocyte lysate system as described by Pelhman and Jackson(22). The assay system contained per ml final volume : 40 µg poly(A)+RNA and 300 µCi L ^{35}S Methionine (750-930 Ci/mmol, the Radiochemical Centre Amersham England). The incubation was for 1-hour at 30°C. The reaction was stopped by adding NP-40, 2%.

Isolation and detection of T-antigens.

T-antigen specific proteins labeled with ^{35}S-methionine were isolated from the bulk of proteins synthesized in vitro by immunoprecipitation with serum from hamsters bearing SV40 induced tumor (anti-T serum). The immunoprecipitate was isolated by immunoaffinity chromatography on staphylococcal protein A-sepharose, (23) denatured, and analyzed on SDS-polyacrylamide gels, (6.2, 12.5 or 14 %) as described by Laemmli (24). Electrophoresis was carried out at room temperature at 20 mA for 5-7 hours. The gels were stained with Coomassie blue, destained, vacuum-dried and exposed to Kodirex X-ray films for from 2 to 6 days. The following molecular weight standards were used to calibrate the gels : phosphorylase a (94 K), bovine serum albumin (68 K), catalase (60 K), glutamate dehydrogenase (53 K), ovalbumin (43 K), aldolase (40 K), chymotrypsinogen A (25.7 K), trypsin inhibitor (21.5. K), β lactoglobulin (18,4 K) and cytochrome C (12.5 K). In order to quantitate the amount of individual products synthesized, regions of the gel corresponding to the bands of radioactive small-t and large-T antigens were

cut out from the slab, put into scintillation vials, dissolved with 0.5 ml of NCS solubilizer (Nuclear Chicago) and after the addition of a toluene based scintillation fluid, counted in a SL-30 Intertechnique spectrometer.

Translation of gradient fractionated infected BMK cell mRNA

Poly(A)+cytoplasmic RNA samples (each isolated from 40 petri dishes) in 200 µl of a buffer containing 10 mM triethanolamine, pH 7.4, 50 mM NaCl and 1 mM EDTA (used for preparing sucrose gradients) were centrifuged in 16 ml, 15-30 % (w/v) sucrose gradients in a Spinco SW 27.1 rotor at 23,000 rpm for 20 hr at 20°C. 0.4 ml fractions were collected from the top of the tubes with an ISCO 640 density gradient fractionator while absorbance at 254 nm was monitored with an UA-4 absorbance monitor. Each fraction was precipitated with two volumes of ethanol after addition of NaCl to a concentration of 0.15 M and of 4 µg of 7 S - 4 S RNA isolated from control BMK cell cultures. After ethanol precipitation, the RNA from each fraction was pelleted, dissolved in 25 µl H_2O, and stored at -20°C. The reticulocyte lysate system was programmed with 5 µl RNA from each fraction, the translation products were immunoprecipitated with control and anti-T serum, and analysis was carried out by gel electrophoresis, as described above.

RESULTS

A- In vitro translation of poly(A)$^+$ cytoplasmic RNA from cells infected with SV40 WT and dl 54/59 mutants.

Using the wheat germ system, mRNA extracted from SV40-infected monkey kidney cells was shown to direct the cell-free synthesis of two polypeptides which could be specifically immunoprecipitated with anti-T serum, and had respectively the same mobility as the large-T and small-t antigens synthesized *in vivo* (2, 5). Recently we have also demonstrated the synthesis of these two polypeptides in the reticulocyte lysate system in response to mRNA (4) extracted from cells that were either abortively or productively infected with SV40, and from a variety of transformed cell lines.

In the present study, we first compared large-T and small-t antigens synthesized in the reticulocyte lysate system under the direction of the poly(A)+cytoplasmic RNA extracted from BMK cells abortively infected with either wild type SV40 or with dl 54/59 mutants 2102, 2112, 2122, 891 and 883. The location and size of the deletions in these mutants (6, 8, 10) are shown in Table I and Fig. 1. Poly(A)+cytoplasmic RNAs were isolated from SV40-infected BMK cells 12 hours postinfection, and translated in the reticulo-

TABLE I

EFFECT OF DELETIONS (54/59) ON THE APPARENT MOLECULAR WEIGHT OF SV40 SMALL-t PROTEIN

Mutant	Map position	Deletion size (base pairs)	Apparent MW (kilodaltons) of small-t translation product from	
			Poly(A)+cytoplasmic RNA	Poly(A)+nuclear RNA
dl 2102 [x]	0.565	- 30	18.5	18.5
dl 2122 [x]	0.55 - 0.60	-250	17	17 and 11
dl 2112 [x]	0.54 - 0.60	-310	no small-t detected	11
dl 883 [xx]	0.54	- 23	no small-t detected	18.5 (+)
dl 891 [xx]	0.59	- 41	12	12
WT (strain 776 or LP)			19	19

x map position and deletion size according to Feunteun et al. (1978)

xx map position and deletion size according to Crawford et al. (1978)

(+) faint band.

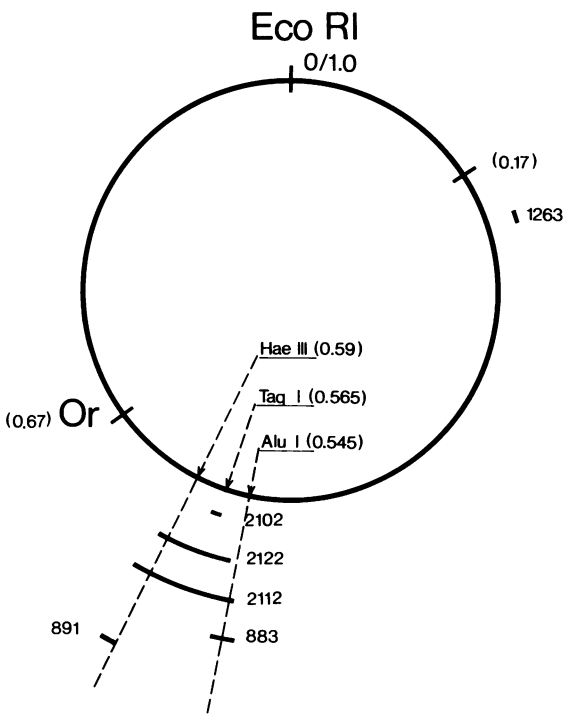


Fig. 1 : Map location and size of the SV40 deletion mutants used in the present work.

cyte lysate system. The in vitro translation products were immunoprecipitated with control and anti-T serum. The immunoprecipitates were isolated with protein A - sepharose and analyzed by polyacrylamide gel electrophoresis followed by autoradiography. Fig. (2) shows that translation of the mutant RNAs produce normal-sized large-T antigen but either produces a small-t antigen which is reduced in size, or fails to produce any detectable small-t. The apparent molecular weights of the small-t polypeptides synthesized in vitro by SV 40 wild type and dl 54/59 mutants are shown in Table I. Very similar results have been obtained with the corresponding large-T and small-t polypeptides extracted from SV40-infected cells for the wild type, mutants dl 2102, 2122, 891 (8). In the case of mutants dl 2112 and 883, there is no detectable small-t either in the translation products synthesized in response to poly(A)+cytoplasmic RNA (Fig. 2 and 3 a ; Table I) or in extracts from infected cells (for dl 2112 : réf. 8 ; for dl 883 : May, unpublished ; Crawford, personal communication). The question of what prevents the production of small-t or polypeptide related to small-t by these two mutants will be examined below (see part C).

B - Characterization of the mRNAs coding for small-t and large-T

- Comparison of the sedimentation patterns of large-T and small-t mRNAs in aqueous sucrose gradient.

Poly(A)+cytoplasmic RNA from SV40-infected BMK was fractionated on aqueous sucrose gradients. Gradient fractions were collected, precipitated and translated in the reticulocyte system ; the translation products were immunoprecipitated with control and anti-T serum and analyzed on SDS-polyacrylamide gel, as described in Materials and Methods (Fig. 4, a). Large-T and small-t antigens were located on the gel by autoradiography, and the amount of radioactivity present in appropriate gel slices was determined. The sedimentation profiles of the mRNAs coding for large-T and small-t antigens were obtained by plotting the amount of radioactivity contained in the corresponding bands (T or t) against gradient fraction number (Fig. 4, b). Fig. 4 (a) shows, as previously reported by Paucha et al. (5) that the mRNA coding for small-t sediments to the heavy side of that coding for large-T. This observation is illustrated in Fig. 4 (b) by a graphic representation ; the peak of small-t mRNA is one fraction ahead of the peak of large-T mRNA, both peaks corresponding to a sedimentation velocity of approximately 19 S. This result is consistent with the notion that the size of small-t mRNA is slightly larger than that of large-T mRNA.

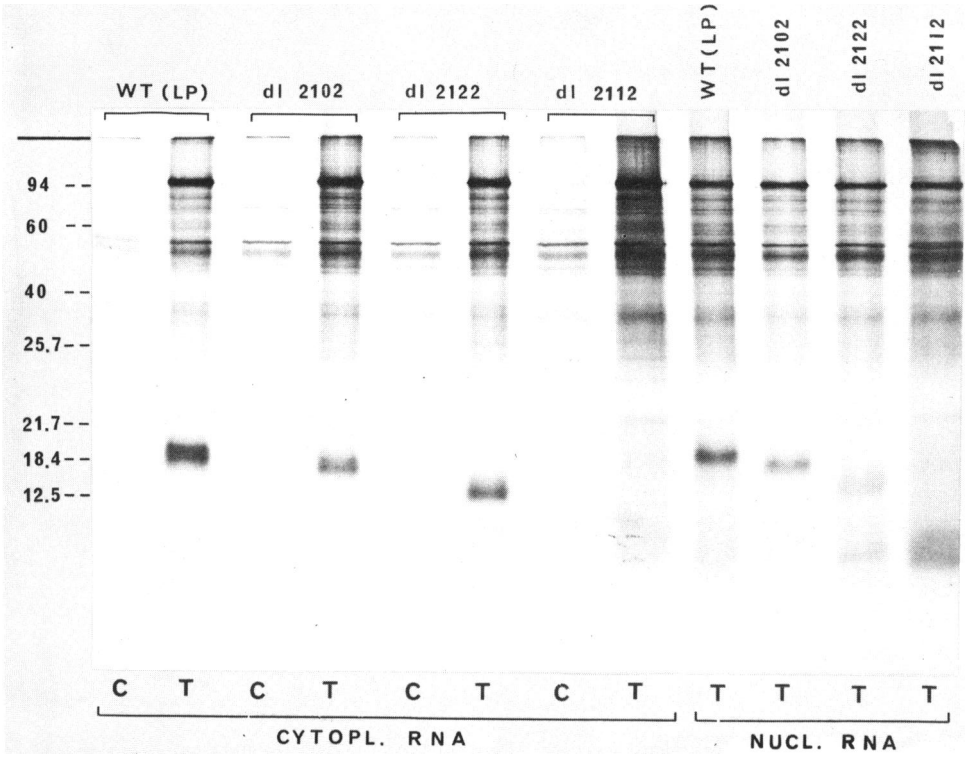
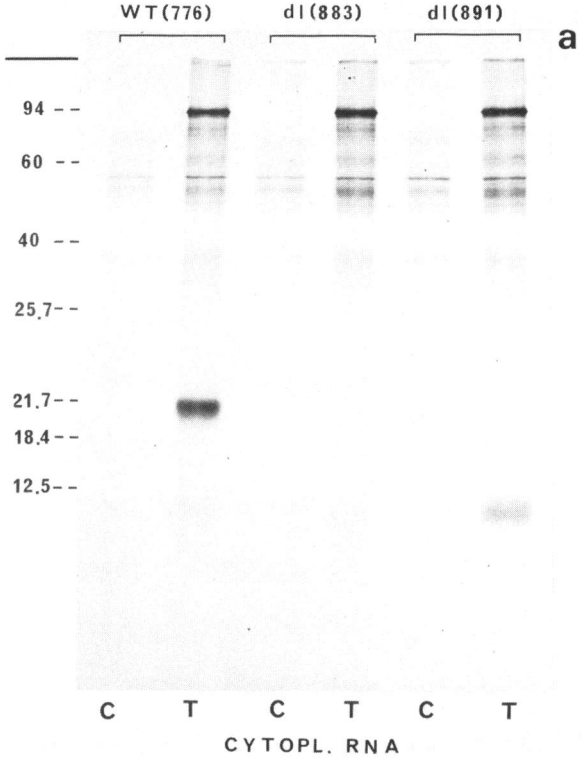


Fig. 2 : Anti-T immunoprecipitable proteins made in response to cytoplasmic and nuclear poly(A)+RNA from BMK cells infected with dl 2102, dl 2122, dl 2112 or their parental wild-type SV40 (LP strain). Cytoplasmic and nuclear poly(A)+RNA from infected cells were translated in the reticulocyte system, and the products were immunoprecipitated with control (C) and anti-T (T) serum. The immunoprecipitates were isolated and analyzed by 14 % SDS-polyacrylamide gel electrophoresis followed by autoradiography. The left hand eight tracks show the translation products from poly(A)+cytoplasmic RNA; the right hand four tracks show the translation products from poly(A)+nuclear RNA. The figures on the left indicate the positions of the molecular weight markers (apparent MW in kilodaltons).



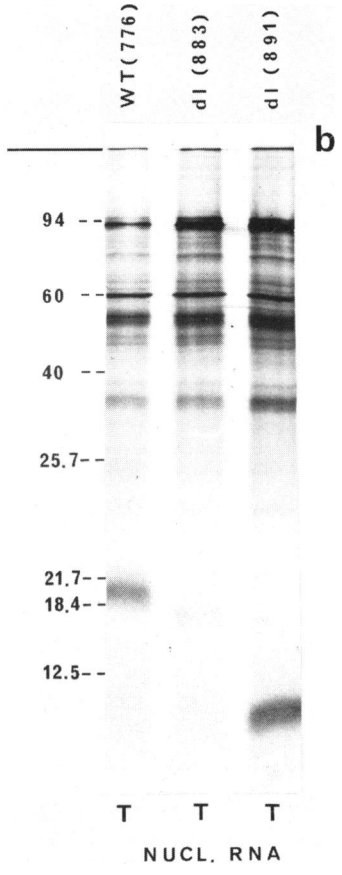


Fig. 3 : Anti-T immunoprecipitable proteins made in response to cytoplasmic and nuclear poly(A)+RNA from BMK cells infected with dl 883, dl 891 or their parental wild-type (strain 776). Same as in legend Fig. 2 except that the viruses used were dl 883, dl 891, and their parental wild type (strain 776). Translation products from poly(A)+cytoplasmic RNA (panel a) and from poly(A)+nuclear RNA (panel b).

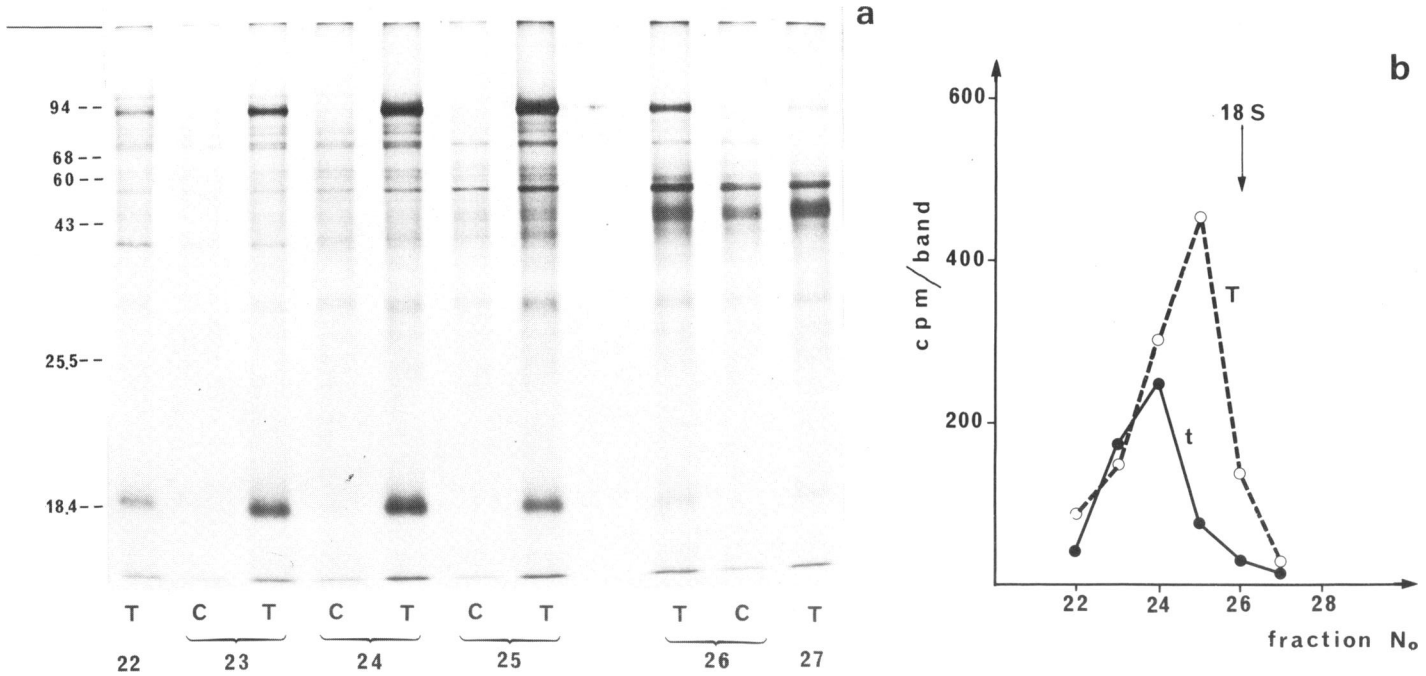


Fig. 4 : Sedimentation profiles of SV40 early mRNA for small-t and large-T. Panel a : Poly(A)+cytoplasmic RNA from SV40-infected BMK cells was fractionated on (16ml) linear (15-30 % w/v) sucrose gradient by centrifugation in Spinco 27.1 rotor at 23 000 rpm for 20 hr at 20°C. Forty 0.4ml fractions were collected. The RNA from each fraction was ethanol-precipitated in the presence of 4 µg of carrier RNA and redissolved in 25 µl H_2O. The reticulocyte lysate system was programmed with 5 µl RNA from each fraction, the translation products were immunoprecipitated with anti-T (T) serum (and with control (C) hamster serum for comparison) and analyzed on 12.5 % SDS-polyacrylamide gel electrophoresis. Sedimentation was from right to left. The figures on the left indicate the positions of the molecular weight markers (apparent MW in kilodaltons). Panel b : The radioactivity in the bands corresponding to small-t and large-T was counted as described in the text and plotted against fraction number. The arrow indicates the position of 18 S rRNA sedimented in a parallel gradient. ●, small-t ; O, large-T.

- Effect of dl 54/59 mutation on the size of small-t and large-T

The previous results provide evidence that part of the coding sequences for small-t but not for large-T antigen lie between coordinates 0.54 and 0.59. In order to test this hypothesis we examined the effect of the 250 base-pair deletion dl 2122 on the size of small-t mRNA and of large-T mRNA.

Comparison of the sedimentation patterns in sucrose gradient of small-t mRNAs from wild type and dl 2122. A mixture of poly(A)+cytoplasmic RNA preparations obtained separately from SV40 wild type-infected BMK cells (20 dishes) and from mutant dl 2122-infected BMK cells (20 dishes), was fractionated on sucrose density gradients and translated in vitro. The translation products were immunoprecipitated with anti-T serum and the immunoprecipitates were analyzed on 14 % SDS-polyacrylamide gels as described in Materials and Methods. The results are shown in Fig. 5 (a and b). On each track of the gel (Fig. 5, a) the two bands corresponding respectively to the small-t proteins from SV40 wild type and mutant dl 2122 were well separated ; this resolution allowed us to compare the sedimentation profiles of the corresponding mRNAs. Fig. 5 (b) clearly shows that the peak of small-t mRNA from dl 2122 lags one fraction behind that of small-t mRNA from wild type.

Comparison of the sedimentation patterns in sucrose gradient of large-T mRNAs from WT, dl 1263 and dl 2122. It is not possible by this approach to compare the sedimentation patterns of large-T mRNAs from WT and dl-2122 in the same gradient since WT and dl-2122 produce large-T proteins having the same apparent MW. This difficulty may be overcome by using the additional deletion mutant 1263 whose deletion of about 50 base-pairs is at coordinate 0.21 (10). Although this mutant has a short deletion, it produces a large-T which is about 6-8 kilodaltons smaller than wild-type large-T (6). Considering the small size of the 1263 deletion, the large-T mRNAs from wild-type and dl-1263 would be expected to have sedimentation patterns which are very close and this is indeed what is observed in the experiment whose results are presented in Figure 6 (a and b). Similarly Fig. 7 (a, b) shows that the mRNAs from dl-2122 and dl-1263 (and therefore from WT) have sedimentation patterns which are practically indistinguishable.

It may be concluded that deletion 2122 does not cause a detectable change in the sedimentation rate of large-T mRNA, whereas it produces a reduction in the sedimentation rate of small-t mRNA.

C - In vitro translation of poly(A)+nuclear RNA from cells infected with SV40 WT and dl 54/59 mutants.

Berk and Sharp (14) have shown that small-t mRNA is spliced; their

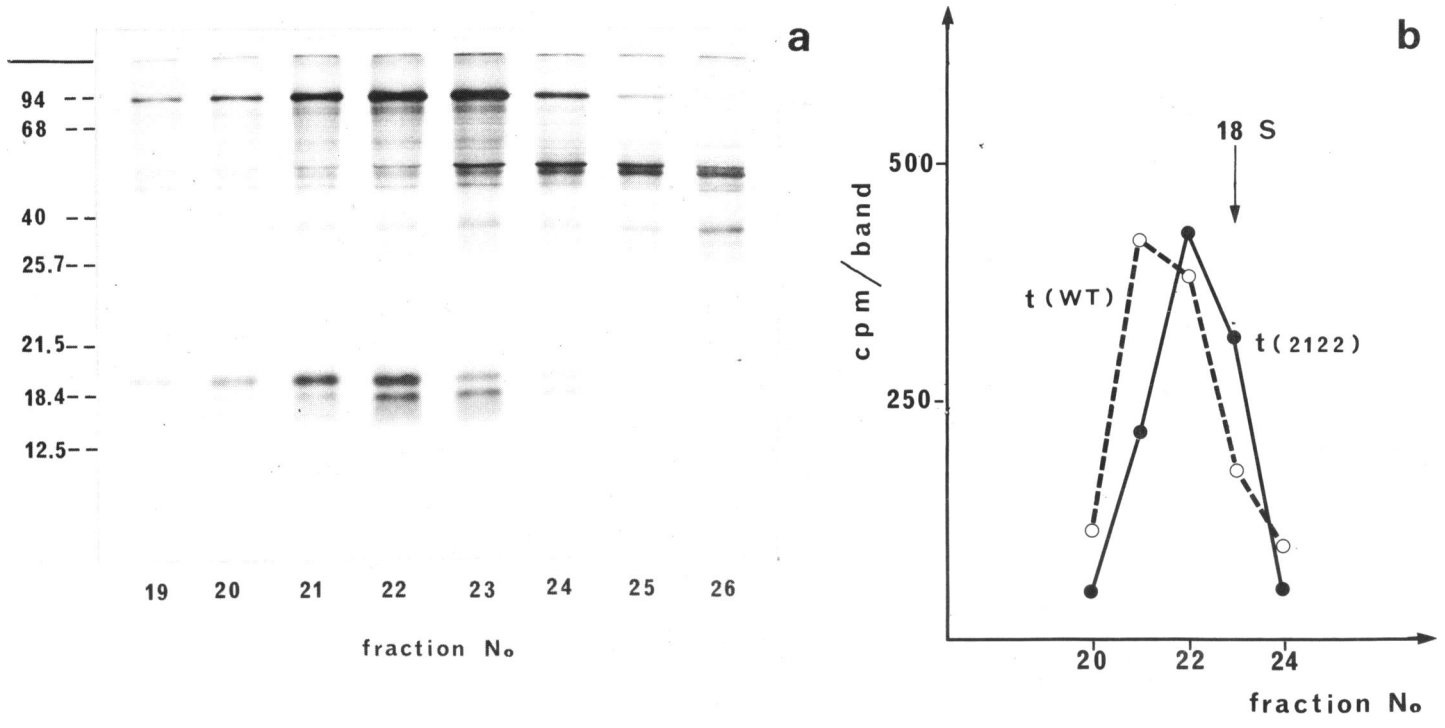


Fig. 5 : Sedimentation profiles of mRNAs coding for SV40 WT small-t and dl 2122 small-t. Panel a : The two poly(A)+cytoplasmic RNA preparations obtained from SV40 wild-type-infected BMK cells and from mutant dl 2122-infected BMK cells were mixed, the mixture was fractionated on a sucrose gradient, and translated *in vitro* ; the translation products were immunoprecipitated with anti-T serum and the immunoprecipitates were analyzed on 14% SDS-polyacrylamide gel. SV40 WT was the LP strain. Sedimentation was from right to left. The figures on the left indicate the positions of the molecular weight markers (apparent MW in kilodaltons). Panel b : The amounts of radioactivity contained in the bands corresponding to small-t from WT and from dl 2122 were counted as described in text and plotted against fraction number. ●, dl 2122; ○, WT.

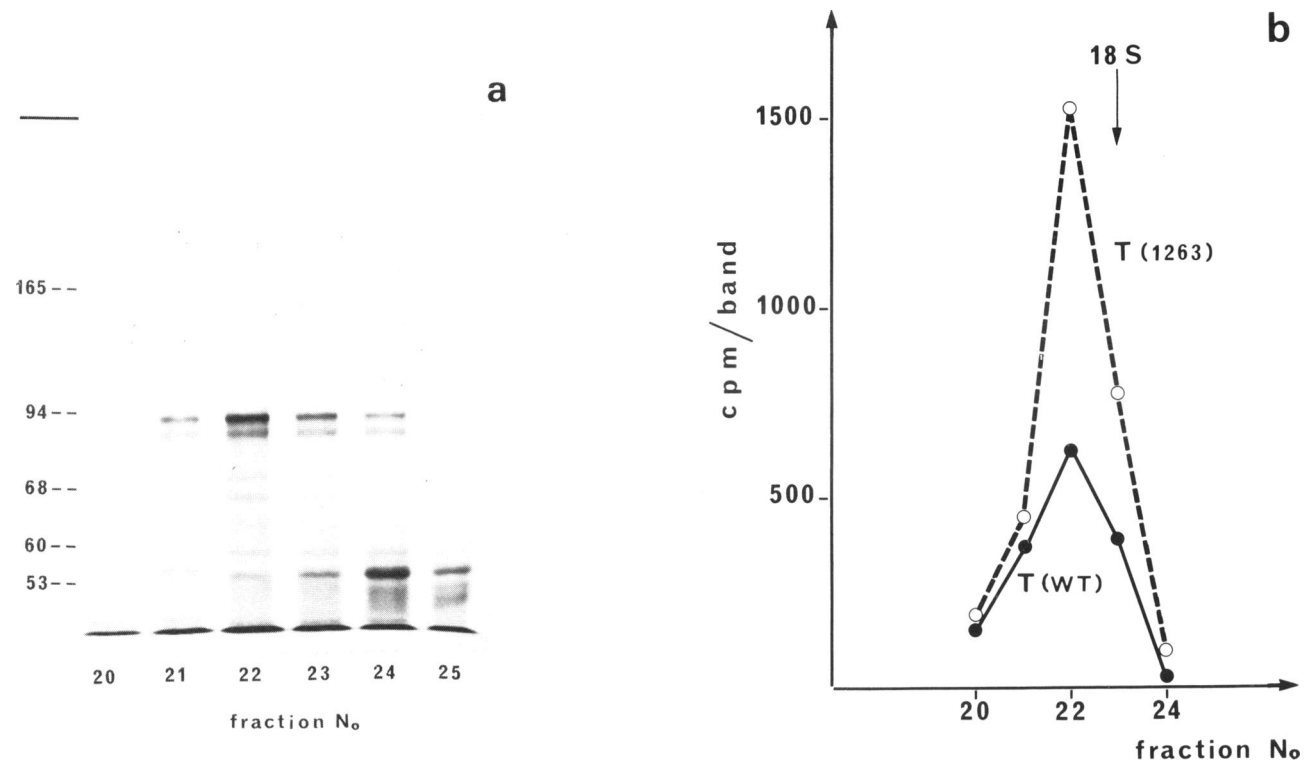


Fig. 6 : Sedimentation profiles of large-T mRNAs from SV40 wild-type (LP strain) and d1 1263 mutant. Panel a : The two poly(A)+cytoplasmic RNA preparations obtained respectively from SV40 wild-type-infected BMK cells and from mutant d1 1263-infected BMK cells were mixed and the mixture was fractionated on sucrose gradient and translated in vitro ; the translation products were immunoprecipitated and analyzed on 6.2 % SDS-polyacrylamide gel. Sedimentation was from right to left. The figures on the left indicate the positions of the molecular weight markers (apparent MW in kilodaltons). Panel b : The radioactivity contained in the bands corresponding to large-T from WT and d1 1263 was counted as described in text and plotted against fraction number. ●, WT ; 0, d1 1263.

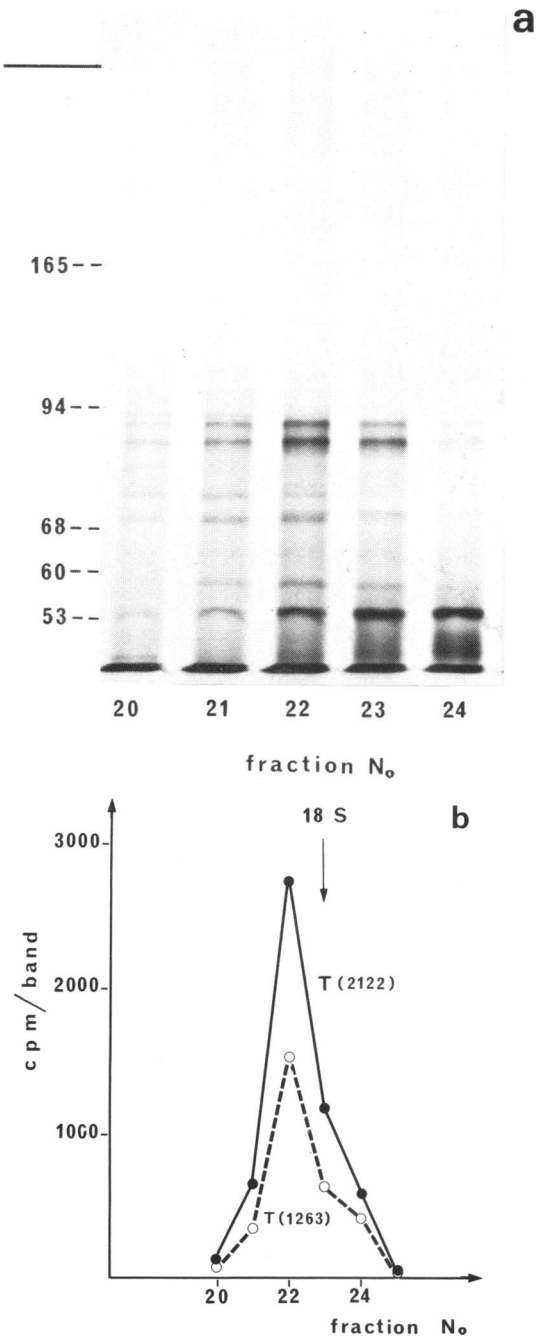


Fig. 7 : Sedimentation profiles of large-T mRNAs from dl 1263 and dl 2122 mutants. Panel a and b : Same experiment as in Fig. 6, except that the two RNA preparations were obtained from BMK cells infected respectively with dl 1263 and dl 2122 mutants.

results suggest that a 50 nucleotide segment is excised from the region around the site 0.545 which corresponds to the Alu B/C junction. The fact that the two mutants (dl 2112 and dl 883) which fail to produce small-t proteins are precisely those which have lost the Alu B/C junction (8) suggests that these mutants may have lost one of the sites at which splicing normally occurs to yield the small-t mRNA. Furthermore, the fact that the three reading frames beyond the regions which are affected by deletions are blocked by a number of termination codons (15, 16) suggests that the "prespliced" early transcript of these mutants might be translated in vitro to give a derivative of small-t. To test this hypothesis, we examined the in vitro translation products synthesized in response to poly(A)+nuclear RNA from BMK cells infected with SV40 WT or dl 54/59 mutants.

The poly(A)+nuclear RNA was obtained from the same set of infected BMK cultures which were used in the experiment reported in part A. The RNA was translated in the reticulocyte system, the translation products were immunoprecipitated with control and anti-T serum and the immunoprecipitates were analyzed on 14% SDS-polyacrylamide gels as described in Materials and Methods. The results are presented in Fig. 2, 3b and Table I. In the case of dl 2102 and dl 891, the anti-T immunoprecipitable polypeptides synthesized under these conditions consist of (i) a protein comigrating with, and probably similar to large-T, (ii) a protein comigrating with, and probably similar to the corresponding small-t related polypeptide. In these experiments, caution must be used in interpreting the presence of polypeptides which are similar to those obtained by in vitro translation of poly(A)+cytoplasmic RNA, since the possibility that traces of cytoplasm contaminate the nuclear preparations cannot be ruled out.

The case of mutants dl 2112, dl 883 must be examined in more detail. The nuclear RNA from the cells infected with the mutants dl 2112 and dl 883 directs in vitro, the synthesis of both an apparently normal large-T, and a polypeptide within the range of mobility of small-t protein. The apparent MW of this last polypeptide is 11 K for dl 2112, and 18.5 K for dl 883. The 18.5 K band obtained with dl 883 is rather faint, in contrast to the dark 11K band obtained with dl 2112. Taking into account the recent data obtained on the nucleotide sequences of these mutants (Volckaert, Feunteun and Fiers, in preparation), the molecular weights of these polypeptides are compatible with the expected MW of the translation product of the SV40 "prespliced" early transcript RNAs, read from the normal initiation codon to the first termination codon encountered. These polypeptides should contain the N-termi-

nal sequences common to small-t and large-T. In order to test this hypothesis the analysis of the tryptic peptides of these polypeptides, and of WT small-t and large-T is in progress.

The case of dl 2122 is probably the most remarkable. Whereas this mutant produces, both in vivo (8) and by in vitro translation of cytoplasmic RNA, a small-t derivative larger (apparent MW : 17 K) than that expected from the size of the deletion, the poly(A)+nuclear RNA directs the synthesis of two small-t derivatives. The size of the smaller derivative (apparent MW 11K) is compatible with the size of the deletion ; and the larger (apparent MW 17 K) appears to be similar to the in vivo product. It is noteworthy that SV40 DNA I from the preparations of dl 2122 used in the present study gave, by restriction enzyme analysis, the typical cleavage pattern expected for this mutant (J. Feunteun, personal communication), ruling out the possiblity of these preparations being contaminated.

DISCUSSION

Characterization of two SV40 early mRNAs

In the present study, it was first shown, by using the in vitro translation of gradient fractionated poly(A)+cytoplasmic RNA from SV40 infected cells, that the sedimentation profiles of the mRNA coding for small-t and large-T could be resolved, and that the dl 54/59 mutation 2122 detectably reduced the sedimentation velocity of small-t mRNA, but not that of large-T mRNA. These results lend support to the suggested model that the mRNAs for small-t and large-T are distinct, and that the region 0.59 to 0.54 of the viral genome is represented in the sequence of small-t mRNA, but not in that of large-T mRNA(6, 7, 8, 9, 14, 16).

Evidence for a nuclear "prespliced" RNA species

We have shown that the translation of the poly(A)+nuclear RNA of mutants dl 883 and dl 2112 which make no small-t in vivo (or by in vitro translation of cytoplasmic RNA) produces a low molecular weight polypeptide immunoprecipitable with anti-T serum. These results are consistent with the hypothesis that deletions 883 and 2112 prevent the splicing which normally yields small-t mRNA, and that the low MW polypeptide synthesized in vitro in response to poly(A)+nuclear RNA is the translation product of the SV40 "prespliced" early RNA. This hypothesis suggests (i) that the mRNA splicing occurs concurrently with, or after the polyadenylation of the nuclear precursor and (ii) that the splicing of small-t mRNA occurs in the nucleus and is a controlling step for the nucleocytoplasmic transfer of small-t mRNA. Similar

conclusions have been reached from the analysis of SV40 late mRNA synthesis (25; Lai and Khoury, in preparation).

In the case of mutant dl 2122, Feunteun et al. (8) have already discussed the observation that the 17 K small-t related polypeptide produced in vivo by this mutant seems too large to be accounted for by an "in-phase" deletion. This mutant also produces another small-t derivative (MW: 11K) in the nuclei. Nucleotide sequence data which have recently been obtained on dl 2122 (Volckaert, Feunteun and Fiers, in preparation) show that the deletion is indeed not in-phase. Our present data can be interpreted as follows: (i) the 11 K protein probably represents the translation product of the nuclear "prespliced" early RNA read from the normal initiation codon to a termination codon located near the upstream junction of the small-t mRNA splice; and, (ii) since the termination codon functioning in the prespliced RNA is not present in the spliced small-t mRNA, translation of this altered mRNA could proceed further beyond the splice site in a frame different from that of the large-T, giving the 17 K protein.

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