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Altered Protein Metabolism in Infection by the Late tsB11 Mutant of Simian Virus 40

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The DNA of the temperature-sensitive mutant tsB11 is replicated at the same rate as the DNA of wild-type virus in infection at the restrictive temperature. The progeny mutant DNA cannot be distinguished from wild-type DNA by gel electrophoresis and is assembled into a nucleoprotein complex with the same velocity sedimentation characteristics as the wild-type complex. Analysis of in vivo protein synthesis by sodium dodecyl sulfate polyacrylamide gel electrophoresis and immunoprecipitation techniques demonstrated that the capsid components VP1, VP2, and VP3 of the mutant and wild-type virus are synthesized at a similar rate, but VP1 fails to accumulate within cells infected by tsB11. Furthermore, VP1 is located predominantly in the cytoplasmic rather than in the nuclear fraction of extracts from cells infected by the mutant. Immunofluorescent studies localized virion antigen within the nucleolus as well as the cytoplasm. The altered intracellular distribution and stability of VP1 suggest that it may be the mutant protein of tsB11. The synthesis of a 72,000 dalton protein is consistently induced in significant quantity in cells infected by tsB11 at the restrictive temperature. A protein of the same apparent molecular weight is present in smaller quantities in uninfected cells and is only slightly increased in quantity in cells infected by wild-type virus.

Temperature-sensitive (ts) mutants of simian virus 40 (SV40) have been isolated to define the functions of each viral gene and the interrelationships between the viral genes (3, 5, 18, 19, 21, 24). Mutations affecting a wide range of phenotypic manifestations both early and late in infection have been identified. The early mutants, but not the late mutants, are defective in the synthesis of infectious viral DNA in monkey kidney cells and in the transformation of mouse cells (18, 19, 21). Most of the late mutants produce viral structural antigens and defective viral particles (24). A few late mutants, including tsB11, are temperature-sensitive in the synthesis of stable viral structural antigen, capsomeres, and particles (16, 24).

Thus tsB11 may provide an opportunity to study the assembly of the SV40 virion in vivo and in vitro. However, additional characteristics of this mutant distinguish it from wild-type virus and most of the other late mutants. The early U antigen (14) cannot be detected in infection by tsB11 at 41 C (20), yet the mutation maps exlusively in the late G region of the SV40 genome (C. J. Lai and D. Nathans, Virology, in press). We undertook these studies to localize the defective function of tsB11. Based on previous data, the mutation could theoretically have affected the completion of viral DNA replication, the transcription or processing of viral RNA, the translation or cleavage of viral proteins, or the location and fate of viral proteins after synthesis.

We will show that the viral capsid proteins are synthesized at the restrictive temperature, but the intracellular distribution and stability of the major capsid protein in the cell is clearly altered. In addition, the unexpected accumulation of a 72,000 dalton protein in tsB11-infected cells suggests the existence of a step in virus propagation which has not previously been appreciated.

MATERIALS AND METHODS

Cell culture. The TC7 clone (17) of the CV-1 line of monkey kidney cells was cultivated in Eagle medium containing 5 to 10% fetal bovine serum (FBS) buffered at pH 7.4 with either $NaCHO_3$-CO_2 or 80 mM Tricine (Sigma Chemical Co., St. Louis, Mo.).

Virus. Wild-type (WT) SV40 was derived from the small-plaque VA 45-54 strain by cloning (22). Mutants tsB4 and 11 were derived from the WT clone after intracellular mutagenesis with nitrosoguanidine

(22). Mutant tsA30 was isolated after extracellular exposure of WT virus to hydroxylamine (21). WT and mutant stocks were grown at 33 C after inoculation of monolayer TC7 cultures at an input multiplicity of 0.01 PFU per cell and were titered at 33 and 41 C as previously described (17, 22).

Buffers. Tris-buffered saline (TBS) is 137 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.7 mM Na_2HPO_4, and 25 mM Tris-hydrochloride, pH 7.4. TBS without calcium and magnesium is designated TD buffer. Tris-EDTA buffer (TE) is 0.01 M Tris-hydrochloride and 0.01 M EDTA, pH 7.4. Phosphate buffer (NaP) is 0.01 M $NaH_2PO_4 \cdot Na_2HPO_4$, pH 7.2.

Isotopes. Radioisotopes were obtained from New England Nuclear Corp. DNA was labeled with either [^{3}H]thymidine (40 to 60 Ci/mM) or [^{14}C]thymidine (0.05 Ci/mM). Protein was labeled with [^{35}S]methionine (40 to 100 Ci/mM).

Infection. Confluent TC7 monolayers in 8-oz. prescription bottles (45-cm^2 cell growing area) were inoculated with input multiplicities of 10 PFU per cell. Mock infection was carried out in the same way with lysates from uninfected cells. After a 2-h absorption period at room temperature, the inoculum was replaced with medium containing 5% FBS.

SV40 DNA. Viral DNA was selectively extracted from infected cells as described by Hirt (11) and dialyzed overnight in TE buffer. Radioisotope-labeled DNA was analyzed by gel electrophoresis by the method of Tegtmeyer and Macasaet (23). Samples (0.1 ml) were subjected to electrophoresis through 1.5% agarose containing 0.2% sodium dodecyl sulfate (SDS) for 2 h at 10 V per 6-cm gel.

SV40 nucleoprotein complexes. Viral nucleoprotein complexes were extracted from infected cells with Triton X-100 as described by Green et al. (10) and White and Eason (26). Velocity sedimentation of the radioisotope-labeled complexes was performed by layering 0.2-ml samples on 4-ml linear 5 to 20% sucrose gradients in 0.01 M Tris, 0.2 M NaCl, 0.01 M EDTA, pH 8.0. The samples were centrifuged for 2 h in an SW65 Spinco rotor at 36,000 rpm at 4 C. Fractions were drop collected, precipitated with 10% Cl_3CCOOH, and assayed for radioactivity in Triton-toluene. Marker SV40 [^{32}P]DNA was obtained from purified virions as previously described (24).

SV40 and cellular proteins. Radioisotope-labeled proteins were extracted from infected and control cells after cellular fractionation as described by Walter et al. (25). Monolayers were washed twice with 10 ml of TBS at 4 C. The nuclei and cytoplasm were separated by adding 1 ml of TBS containing 0.5% Nonidet P-40 (NP-40) and 0.3 mg of phenylmethylsulfonylfluoride (PMSF, Calbiochem) per ml to each culture. After 20 min at 4 C, the samples were centrifuged at 2,000 $\times$ g for 10 min. The cytoplasm in the supernatant fluid was decanted into 1 ml of protein electrophoresis sample buffer containing 0.0625 M Tris, 2% SDS, 5% 2-mercaptoethanol, 30% glycerol, and 0.001% bromophenol blue, pH 6.8. The nuclear pellet was washed twice with 5 ml of TBS at 4 C and resuspended in 1 ml of sample buffer. The cytoplasm and nuclei were then heated at 100 C for 10 min, dialyzed at 4 C in sample buffer overnight, and frozen until analyzed.

Gel electrophoresis of proteins. The protein samples were analyzed by discontinuous SDS polyacrylamide gel electrophoresis as described by Laemmli (13). Separating gels contained 12% acrylamide and 0.15% bis-acrylamide. The well gel was 5% acrylamide and 0.25% bis-acrylamide. The gels were prepared and run on a slab gel apparatus (Hoefer, San Francisco, Calif.). Samples contained 75,000 to 100,000 counts/min in 25 μliters. Electrophoresis was carried out at room temperature for 4 h at 200 mA. The gels were fixed and stained with Coomassie blue according to Fairbanks et al. (7), vacuum dried, and autoradiographed on Kodac Royal X-omat medical X-ray film for 7 to 14 days. Molecular weight markers were obtained from Schwarz-Mann. Capsid protein markers were obtained from purified viral particles labeled with [^{14}C]arginine or [^{14}C]lysine as previously described by Ozer (15). Autoradiograms were scanned with a Joyce-Loebl densitometer to estimate the relative quantities of radiolabel in protein bands. Similar estimates were made by determining the isotope content in bands of the stained gel by liquid scintillation counting.

Immunoprecipitation of viral proteins. After the proteins of interest were identified by autoradiography of the gel slabs, their location in the gel was easily determined by comparing the autoradiogram with the stained dry gel slab. The appropriate bands were carefully removed from the slab with a fine scissors. The protein was eluted from the gel slice by incubation in NaP with 0.5% SDS overnight at 37 C. Portions of the gel fractions (500 to 1,000 counts/min) were diluted to 0.2 ml in 0.5% SDS, 0.5% bovine serum albumin, 0.1 M NaCl, 0.01 M Tris, pH 7.4, and were incubated with 0.02 ml of rabbit antiserum prepared against purified viral particles disrupted with SDS (16). After 60 min at 37 C, 0.2 ml of goat anti-rabbit gamma globulin was added for another 60 min at 37 C. The samples were stored overnight at 4 C, diluted to 5 ml with cold TD, and then centrifuged at 10,000 $\times$ g for 10 min at 4 C. The immune precipitate was washed with 5 ml of cold TD and dissolved in 0.5 ml of 0.5% SDS at 60 C. The radioactive content was determined in Triton-toluene-H_2O.

Immunofluorescent assay and localization of viral protein. Immunofluorescent labeling of viral antigens within infected cells in Microtest plates and the origin of the anti-sera have been previously described (20). The anti-C serum, prepared against capsid proteins isolated on a sucrose gradient after disruption of virions at pH 10.5 (16), reacts by complement fixation with capsid proteins VP1 and VP3 and with intact viral particles. At the concentration used, the anti-V serum, prepared against viral particles, reacts with intact viral capsids but not with individual capsid proteins. None of the antisera reacted with uninfected cells.

RESULTS

Viral DNA synthesis. The replication of viral DNA in TC7 cells infected by WT virus or

tsB11 at 41 C was compared. Infected cells were labeled with [^{3}H]thymidine for 30 min 48 h after infection. Inasmuch as the average duration of a single round of replication at 41 C is 10 to 15 min (23), an accumulation of intermediates by the mutant could be easily detected under these conditions. The viral DNA was selectively extracted and analyzed by gel electrophoresis (Fig. 1). Both viruses produced similar quantities of viral DNA, although the yield of infectious virions of tsB11 was reduced more than 1,000-fold under the same conditons (24). The relative distribution of mutant [^{3}H]DNA into the mature superhelical DNA I, relaxed DNA II, and replicative intermediate components (23) was almost identical to that of WT DNA. Labeling with [^{3}H]thymidine for 24-h periods (not shown) also produced similar gel patterns of WT and mutant DNA.

Viral nucleoprotein complexes. The assembly of nucleoprotein complexes by tsB11 was investigated to localize the defective step in particle assembly more precisely. Cells infected with WT or mutant virus were labeled with [^{3}H]- and [^{14}C]thymidine, respectively, from 24 to 48 h after the onset of infection. A long labeling period was chosen to demonstrate either a defect in primary assembly or an instability of complexes after assembly. Nucleoprotein complexes were extracted with Triton X-100 and analyzed by velocity sedimentation (Fig. 2). Sedimentation of mutant [^{14}C]nucleoprotein at approximately 50*S* was indistinguishable from that of WT [^{3}H]nucleoprotein. Cells infected with WT virus or tsB11 were also labeled for 1 h with [^{3}H]thymidine under identical conditions. No significant quantitative difference in the amount of viral nucleoprotein produced in cells infected by either virus was detected (data not shown).

SV40 and cellular protein synthesis. The patterns of protein synthesis in cells infected by WT and mutant virus were compared. Long periods of labeling with [^{35}S]methionine were used to measure the accumulation and stability

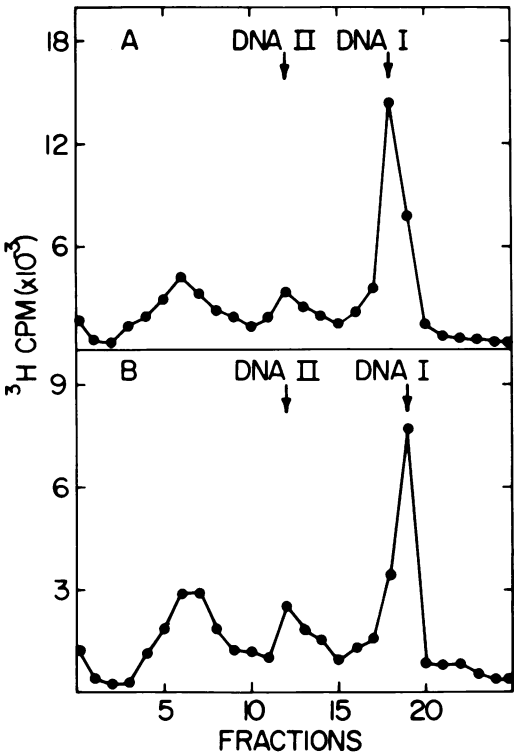

FIG. 1. *Synthesis of viral DNA in TC7 cells infected by WT virus or tsB11 at an input multiplicity of 10 PFU per cell. The infected cells were incubated at 41 C for 48 h and then labeled with 50 μCi of [^{3}H]thymidine per ml of medium for 30 min. Viral DNA was extracted by the Hirt technique and analyzed by electrophoresis through 1.5% agarose gels containing SDS for 2 h at 10 V per gel. A, tsB11 DNA; B, WT DNA. The arrows in the figure indicate the positions of [^{32}P]DNA I and DNA II isolated from purified WT virus and added to the samples.*

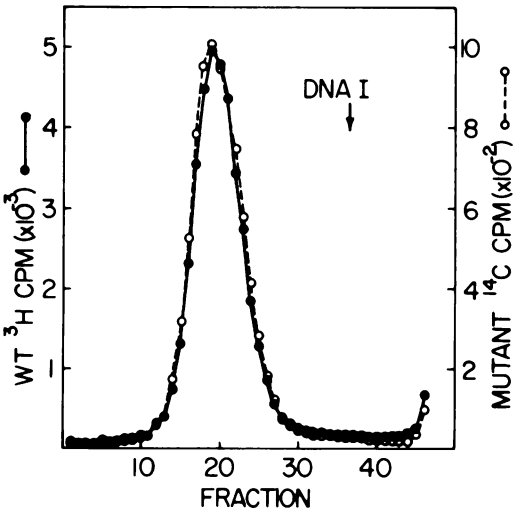

FIG. 2. *Synthesis of viral nucleoprotein complexes in TC7 cells infected by WT virus and tsB11 at an input multiplicity of 10 PFU per cell. After 24 h at 41 C, the cells were labeled with 5 μCi of [^{3}H]thymidine or 1 μCi of [^{14}C]thymidine per ml of medium for 24 h. Triton extracts of the infected cells were mixed together and sedimented through a 5 to 20% sucrose gradient. Fractions were drop collected and numbered in the order of collection. Symbols: ●, WT nucleoprotein; ○, tsB11 nucleoprotein. The arrow in the figure indicates the position of [^{32}P]DNA I.*

of proteins synthesized during infection; short periods of labeling were used to compare rates of synthesis. In addition the cells were separated into nuclear and cytoplasmic fractions to increase resolution and to determine the distribution of the proteins within the cells. Figure 3 shows a molecular weight plot of standard markers and virion proteins (VP) using the buffer system of Laemmli (13) and the slab gel technique.

The gel patterns of nuclear protein extracted from uninfected cells and cells infected by wild-type virus, tsB11, tsB4, or tsA30 after long periods of radiolabeling are shown in Fig. 4. The cells were labeled with [^{35}S]methionine from 48 to 96 h after infection at 33 C and from 24 to 48 h after infection at 41 C. The patterns of proteins synthesized in control cells at 33 and 41 C differed somewhat in the relative quantities of individual proteins produced. Capsid proteins could be identified in the nuclei of cells infected by WT virus or tsB4 by comparing the proteins from infected cells with the proteins of purified SV40 and uninfected cells. In infection by WT virus, VP1 (46,000 daltons) and VP3 (28,000 daltons) were present in similar quantities at 33 and 41 C under the conditions of infection and radiolabeling. VP2 (40,000 daltons) was incompletely resolved from cellular proteins. In contrast, VP1 was clearly reduced in quantity in infection by tsB11 at 41 C but not at 33 C. Furthermore, a 72,000 dalton protein was prominent in the nuclei of the same cells at 41 C. In infection by the early mutant tsA30, no viral structural protein could be detected at the restrictive temperature, as expected.

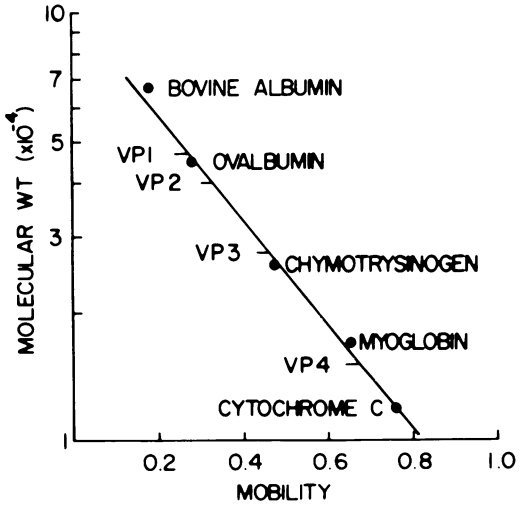

FIG. 3. *Estimation of the molecular weights of SV40 proteins after electrophoresis by the method of Laemmli (10). Standard proteins and SV40 virion proteins labeled with [^{14}C]arginine were subjected to electrophoresis in adjacent wells of an SDS polyacrylamide (12%) gel slab. The positions and mobility of the proteins were determined by staining with Coomassie blue and by autoradiography.*

Selected samples from the autoradiogram shown in Fig. 4 were analyzed on a separate gel and the autoradiogram was scanned with a densitometer to estimate the relative quantities of each capsid protein produced by WT virus and tsB11 at 41 C (Fig. 5). Similar amounts of VP2 and 3 were present in the nuclear extracts of cells infected by either virus. In contrast, VP1 was distinctly reduced in quantity in infection by tsB11 at the restrictive temperature.

The reduced quantity of capsid protein VP1 in tsB11-infected nuclei could have been the result of a reduced rate of synthesis of the protein, instability of the protein after synthesis, or an abnormal intracellular distribution of the protein after synthesis. To distinguish among these possibilities, the gel patterns of proteins from both nuclear and cytoplasmic extracts labeled for either short or long periods at 41 C were examined (Fig. 6, Table 1). Proteins from uninfected cells were similar but not identical after either a 1-h or a 24-h period of radiolabeling. VP1, VP2, and VP3 could again be detected in similar quantities in cells infected by WT virus after either period of labeling. In contrast, the total intracellular content of tsB11 VP1 was significantly reduced after a long but not after a short pulse. These findings indicate that the rate of viral protein synthesis is not reduced in infection by tsB11, but rather the major capsid protein fails to accumulate within infected cells. Furthermore, a greater proportion of VP1 was found in the cytoplasm of tsB11-infected cells than in WT-infected cells. Table 1 quantitates the intracellular accumulation and distribution of VP1 within cells infected by WT virus or tsB11. It should be noted in comparing Fig. 6 and Table 1 that the cytoplasmic extracts were diluted four- to five-fold to facilitate the simultaneous autoradiography of both cytoplasmic and nuclear extracts by using a single slab gel. The quantitation in Table 1 has been corrected by this dilution factor to accurately reflect the intracellular stability and distribution of VP1. No alteration in the cellular location or accumulation of VP2 or 3 could be detected in infection by tsB11.

The 72,000 dalton protein was again induced by tsB11 and was prominent in both nuclear and cytoplasmic extracts. In contrast to VP1, this protein was best demonstrated by a long

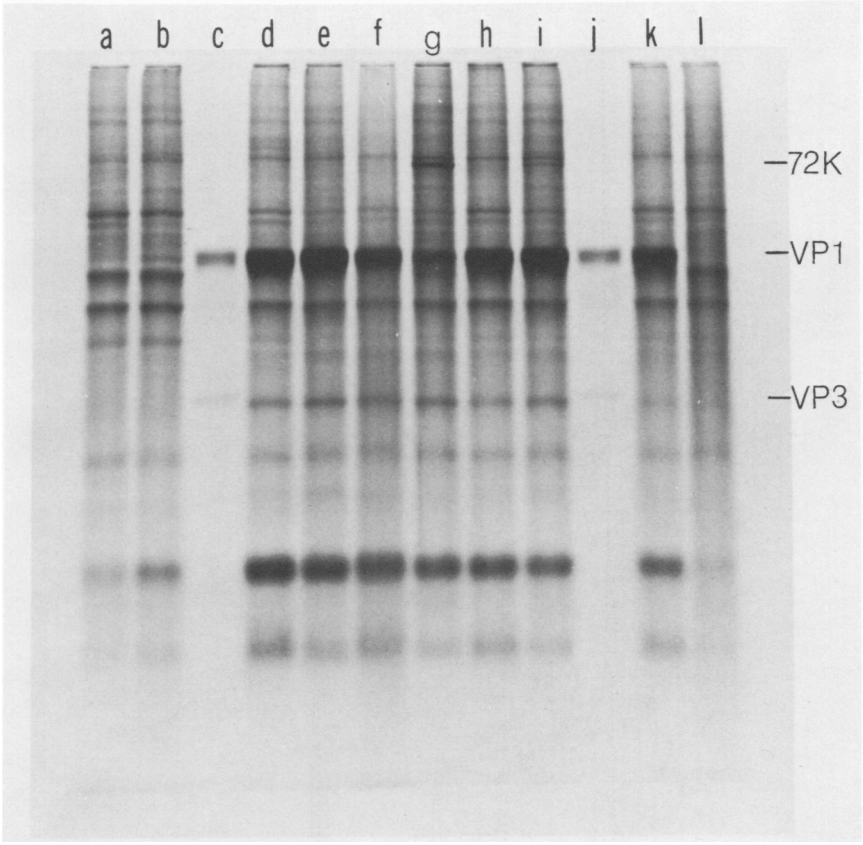

FIG. 4. *SDS polyacrylamide (12%) gel autoradiogram of nuclei extracted from uninfected and infected TC7 cells. The cells were labeled with 5 μCi of [^{35}S]methionine per ml of complete medium 24 to 48 h after infection at 41 C and 48 to 96 h after infection at 33 C. Nuclei were extracted and prepared for analysis as described in Materials and Methods. Approximately 75,000 counts/min were applied to each sample well except c and j. Virion components and relevant virus-induced proteins are labeled at the side margin. The sample order is (a) control cells, 33 C; (b) control cells, 41 C; (c) [^{14}C]leucine-labeled SV40; (d) WT-infected cells, 33 C; (e) WT-infected cells, 41 C; (f) tsB11-infected cells, 33 C; (g) tsB11-infected cells, 41 C; (h) tsB4-infected cells, 33 C; (i) tsB4-infected cells, 41 C; (j) [^{14}C]leucine-labeled SV40; (k) tsA30-infected cells, 33 C; and (l) tsA30-infected cells, 41 C. The densitometer tracings of samples b, e, and g are shown in Fig. 5.*

pulse of radioisotope and is thus relatively stable at 41 C. When the 72,000 dalton protein was labeled for 1 h at 41 C with [^{35}S]methionine, shifted to 33 C, and chased with a 200-fold excess of unlabeled methionine for 2 h, the mutant-induced protein also remained stable at the permissive temperature (Fig. 6). A rapid disappearance of the large protein after the shift to the permissive temperature would have been consistent with the hypothesis that the protein was an uncleaved precursor of the smaller capsid proteins. The relative stability of the protein is inconclusive in this regard. The nuclear and cytoplasmic fractions of uninfected cells contained less densely labeled bands in the same 72,000 dalton region of the gel. WT virus induced the synthesis of this protein only to a limited extent.

In an effort to distinguish between cellular and viral proteins within the gel patterns, individual protein bands were further investigated by immunoprecipitation with rabbit antiserum prepared against disrupted purified empty virus particles. VP1, VP2, and VP3 from cells infected by either WT virus or tsB11 were clearly immunoprecipitated to a significant extent by the specific antiserum (Table 2). The 72,000 dalton protein from tsB11-infected cells did not react with the antiserum to a significant extent.

Localization of viral protein by immunofluorescence. The study of cell fractions by gel

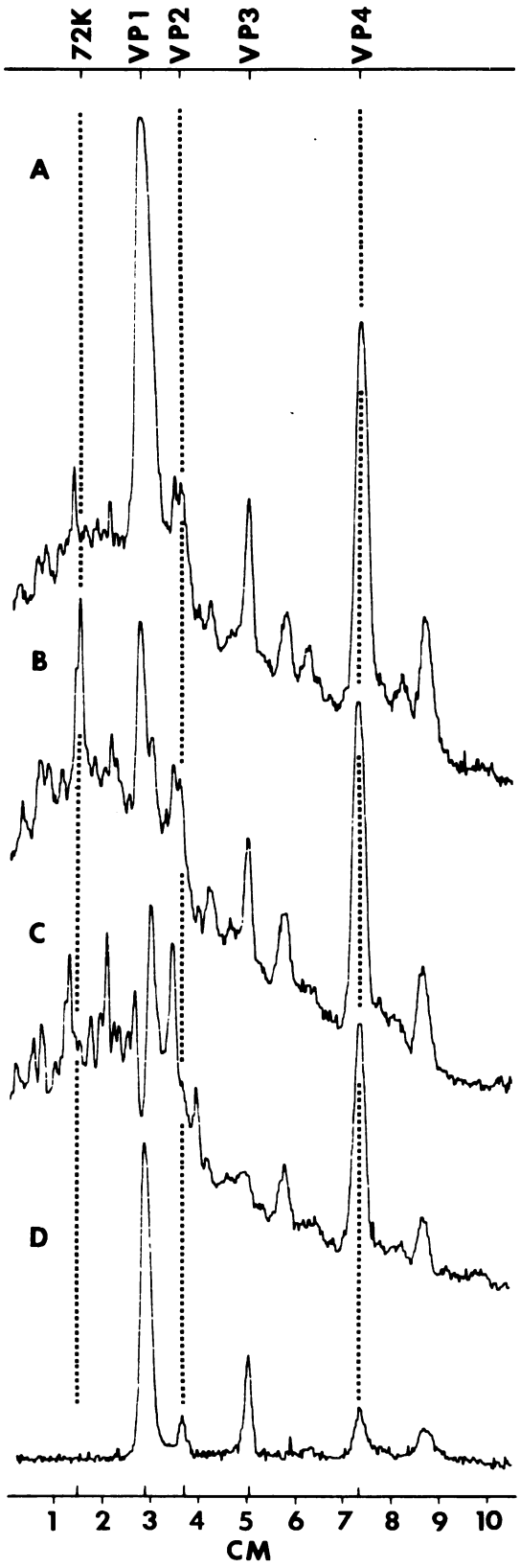

FIG. 5. *Densitometer tracings of an SDS polyacrylamide gel autoradiogram of nuclear extracts. The samples are the same samples used in Fig. 4 but analyzed on a different gel. (A) WT-infected cells, 41 C; (B) tsB11-infected cells, 41 C; (C) control cells, 41 C; and (D) [^{14}C]arginine-labeled SV40. Relevant proteins are labeled at the top of the figure.*

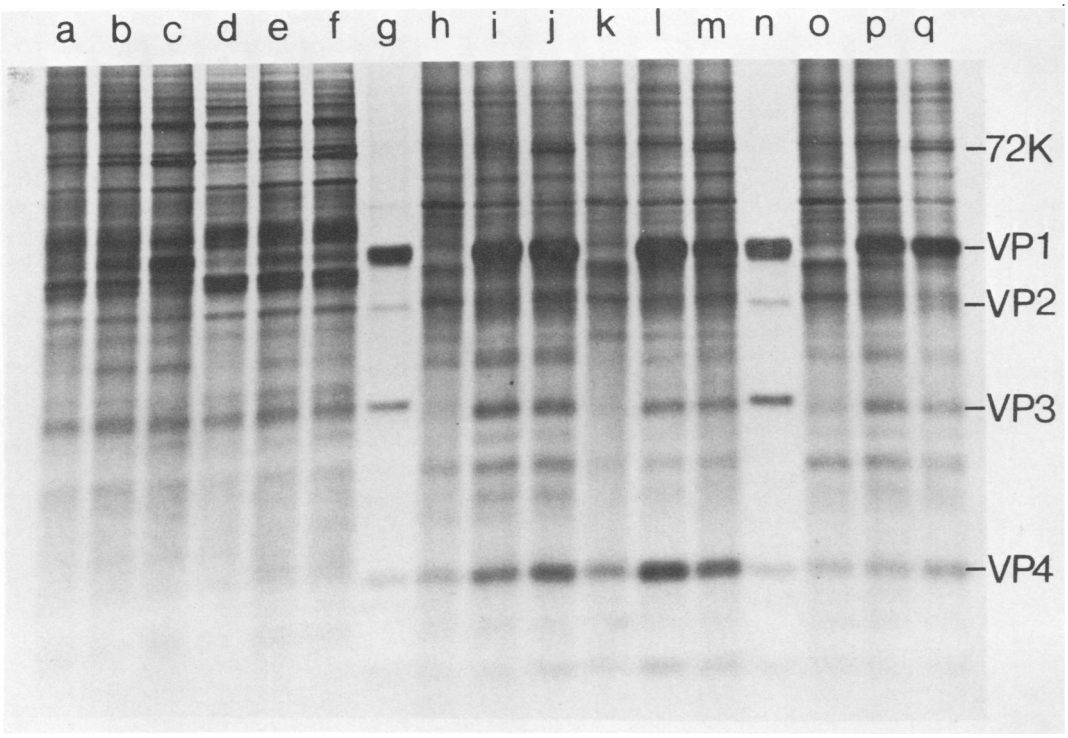

FIG. 6. *SDS polyacrylamide (12%) gel autoradiogram of cytoplasmic and nuclear extracts from uninfected and infected TC7 cells. The cells were labeled for either 1 h with 25 μCi of [^{35}S]methionine per ml of methionine-free medium 47 to 48 h after infection at 41 C or for 24 h with 5 μCi of [^{35}S]methionine per ml of complete medium 24 to 48 h after infection at 41 C. Preliminary experiments had shown that protein synthesis in TC7 cells continues at a uniform rate for 3 h after the addition of methionine-free medium. Approximately 100,000 counts/min were applied to each sample well except g and n. The sample order is (a) cytoplasm, control cells, 1-h pulse; (b) cytoplasm, WT-infected cells, 1-h pulse; (c) cytoplasm, tsB11-infected cells, 1-h pulse; (d) cytoplasm, control cells, 24-h pulse; (e) cytoplasm, WT-infected cells, 24-h pulse; (f) cytoplasm, tsB11-infected cells, 24-h pulse; (g) [^{14}C]arginine-labeled SV40; (h) nuclei, control cells, 1-h pulse; (i) nuclei, WT-infected cells, 1-h pulse; (j) nuclei, tsB11-infected cells, 1-h pulse; (k) nuclei, control cells, 24-h pulse; (l) nuclei, WT-infected cells, 24-h pulse; (m) nuclei, tsB11-infected cells, 24-h pulse; (n) [^{14}C]arginine-labeled SV40; (o) nuclei, control cells, pulse 1 h 41 C, chase 2 h at 33 C; (p) nuclei, WT-infected cells, pulse 1 h at 41 C, chase 2 h at 33 C; and (q) nuclei, tsB11-infected cells, pulse 1 h at 41 C; chase 2 h at 33 C. The top of the gel is not shown in the figure.*

electrophoresis showed that at least one capsid protein (VP1) was altered in its nuclear-cytoplasmic distribution. To localize viral protein without fractionation procedures, infected cells were rapidly fixed and then virus-specific antigens were labeled by indirect immunofluorescence (Fig. 7, Table 3). At 24 or 48 h after infection at 39 C, cells infected by tsB11 demonstrated normal intranuclear T antigen, no detectable V antigen, and an unusual distribution of C antigen limited mainly to the cytoplasm and nucleolus. The pattern was similar at 41 C except that C antigen appeared in the nuclei and nucleoli at 24 h and disappeared from most of the nuclei with a corresponding appearance in the cytoplasm at 48 h. No significant cytoplasmic antigen was detected in cells infected by WT virus under the same conditions. When cultures infected by tsB11 were shifted from 41 C to 33 C and examined 24 h later, the C antigen distribution reverted to that of WT virus within the nucleus.

DISCUSSION

Earlier studies had shown that tsB11 replicated infectious DNA at the restrictive temperature even though the synthesis of infectious virions was reduced more than 1,000-fold (24). However, more recent studies suggested that the "early" U antigen (14) was decreased under the same conditons (20). Inasmuch as relaxed DNA II is infectious, it was important to inves-

TABLE 1. *Intracellular accumulation and distribution of VP1 in TC7 cells at 41 C*[a]

Virus	Radioactivity in VP1 (counts/min)[b]					
	1-h Pulse			24-h Pulse		
	Cytoplasm[c] (Cy)	Nucleus (N)	N/Cy Ratio	Cytoplasm (Cy)	Nucleus (N)	N/Cy Ratio
WT	1,815	3,406	1.9	2,425	4,790	2.0
tsB11	7,350	3,979	0.5	1,670	1,747	1.1

[a] VP1 protein bands were excised from the gels shown in Fig. 6 and dissolved in 30% H_2O_2. The ^{35}S-radioactivity was quantitated by liquid scintillation counting.

[b] Radioactivity present in the portion of the gel of uninfected cells corresponding to VP1 was considered background and subtracted from the total counts in the VP1 region of gels from infected cells. Background radioactivity ranged from 1,077 to 1,215 counts/min.

[c] The cytoplasmic fraction contained approximately five times as many total counts as the nuclear fraction. The radioactivity present in the VP1 area of the gels of the cytoplasm has been multiplied by the appropriate factor to correct for the original dilution of the cytoplasmic sample required for the simultaneous autoradiographic analysis of nuclear and cytoplasmic fractions of the same gel slab.

tigate DNA synthesis by this mutant more extensively. Gel electrophoresis studies have now shown that tsB11 DNA is replicated at a normal rate at 41 C and that the final product has a size, configuration, and stability very similar to that of WT superhelical DNA.

Earlier studies had also shown that stable tsB11 viral particles are not assembled at 41 C (24). Our studies demonstrate the formation of stable nucleoprotein complexes in mutant-infected cells and suggest that the block in assembly occurs at a later step. However, differences in the protein compositon of the large complex might be difficult to detect by velocity sedimentation characteristics alone. Studies by A. Sen, R. Hancock, and A. J. Levine suggest that the nucleoprotein complex of tsB11 has a lower protein/DNA ratio than the WT complex at 41 C after formaldehyde fixation (Virology, in press). A better understanding of the nature of the intracellular SV40 nucleoprotein complex will require a more complete analysis of its individual components.

Numerous studies of the capsid proteins of purified SV40 virions have been reported (1, 2, 6, 8, 9. 12, 15, 25). The estimated molecular weights of the proteins have varied considerably. In general, the molecular weights obtained by electrophoresis with continuous buffer systems have been lower than the estimates with the discontinuous buffer system of Laemmli (13). Recently, the Laemmli method has been used in studies of viral proteins in infected cells because of its superior resolution (1, 12, 25). We have found the appropriate relationship between the mobility and molecular weight of commonly used protein standards (Fig. 3) to justify description of the virus induced proteins on the basis of size by using the discontinuous system. We will attempt to compare our data only with other data obtained with the same method of electrophoresis (1, 12, 25).

VP1, VP2, and VP3 have been identified within nuclear extracts of SV40-infected cells on the basis of the following criteria: (i) the presence of well-defined bands on protein gels of infected but not uninfected cells, (ii) comigration with capsid proteins of highly-purified SV40, (iii) variation in the intracellular distribution and stability of VP1 in cells infected by late mutant tsB11, and (iv) specific immunoprecipitation of the proteins. VP1, VP2, and VP3 could be quantitated within nuclear extracts, but only VP1 was present in sufficient quantity to be clearly identified within cytoplasmic extracts. The differing efficiency of immuno-precipitation between the capsid proteins probably reflects the degree of purity of the protein in the bands excised from the gel rather than the degree of immuno-reactivity of each protein.

Using these methods to study in vivo protein synthesis our findings can be summarized as follows. Under restrictive conditions, the early mutant tsA30 produces no detectable capsid proteins. Late mutants, tsB4 and tsB11, synthesize structural viral proteins at a rate similar to

TABLE 2. *Immuno-precipitation of virus-induced intranuclear proteins by anti-SV40 serum*

Protein (mol wt)	Protein precipitated (%)			
	Uninfected	WT	tsB11	tsA30
72K	5.8	11.4	11.8	7.1
46K (VP1)	9.6	52.1	56.1	6.8
40K (VP2)	8.8	25.6	35.1	3.0
28K (VP3)	13.4	27.2	32.2	4.3

[a] The same samples which are shown in Fig. 4 were separated on preparative 12% SDS-polyacrylamide gels. After staining with Coomassie blue, protein bands were excised and incubated overnight at 37 C in 0.5% SDS in NaP buffer to elute the proteins. Preparation of the eluted proteins and immunoprecipitation techniques are described in Materials and Methods. Serum collected from animals prior to immunization did not precipitate viral proteins above the levels seen in uninfected cells.

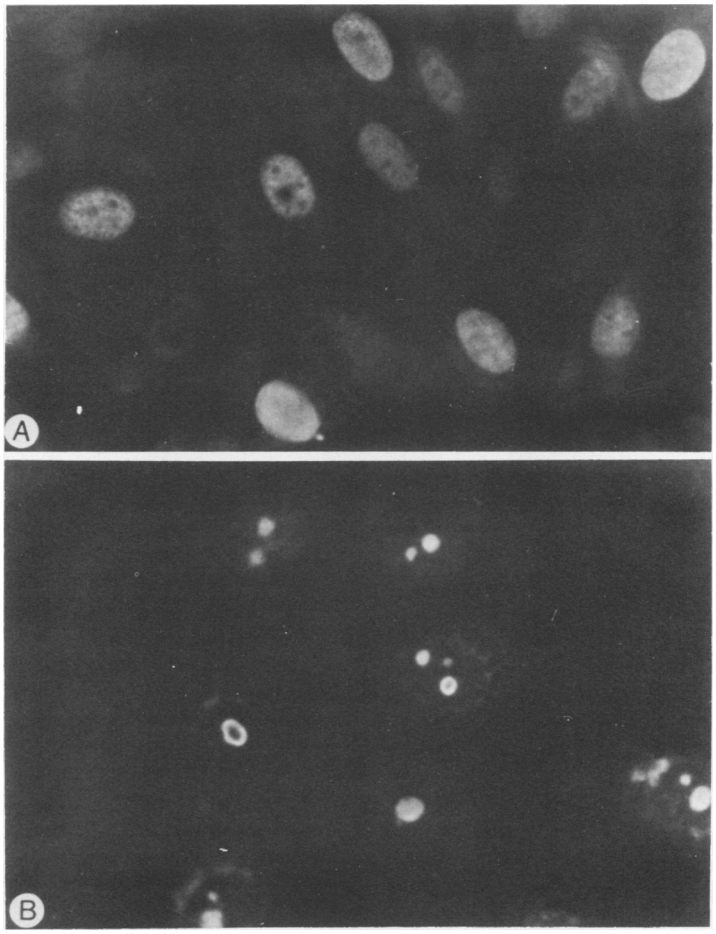

FIG. 7. *Immunofluorescent staining of viral C antigen in TC7 cells infected by tsB11 at an input multiplicity of 1 PFU per ml. A, 72 h after infection at 33 C; B, 48 h after infection at 41 C.*

WT virus. Mutant tsB11 produces unstable VP1 which is located predominantly in the cytoplasm rather than in the nucleus, whereas tsB4 resembles WT virus. Furthermore, the immunofluorescent localization of tsB11 virion antigen in the cytoplasm suggests that the increase of tsB11 VP1 in the cytoplasm is not the result of an artifact of the NP-40 extraction procedure. The abnormal distribution and stability of VP1 could be either the cause or the result of the defective virion assembly previously reported in infection by tsB11 (24). However, transport of VP1 across the nuclear membrane in cells infected by the mutant is probably not defective because immunofluorescence studies localize viral antigen within nuclei early in infection at 41 C. The failure of tsB11 VP1 to accumulate in the infected monolayer is not caused by a loss of cells from the monolayer. Both morphological studies and pulse-chase studies using radiolabeling of protein show no significant loss of cells infected by the mutant from monolayers during the time periods used in these studies (unpublished data). The decrease in labeled VP1 cannot be explained by a redistribution within intracellular compartments because VP1 decreases in both the nucleus and cytoplasm.

Unexpectedly, tsB11 clearly induced the synthesis of a protein with an approximate molecular weight of 72,000 at the restrictive temperature. This protein was consistently found in numerous separate experiments using many different virus pools. In addition the related tsB2 mutant induced the same protein to a similar extent. A faint corresponding band was seen in uninfected cells and was slightly increased in cells infected by WT virus. Other investigators have reported the induction of high molecular weight proteins within extracts of SV40-infected cells. Anderson and Gesteland

TABLE 3. *Intracellular distribution of viral antigens in TC7 cells*

Virus	Antigen-positive cells with nuclear (N), nucleolar (NL), or cytoplasmic (Cy) antigens (%)[a]								
	T antigen			C antigen			V antigen		
	N	NL	Cy	N	NL	Cy	N	NL	Cy
WT									
33, 39, 41 C[b]	100	0	0	100	0	0	100	0	0
tsB11									
33 C	100	0	0	100	0	0	100	0	0
39 C	100	0	0	0	100	100	0	0	0
41 C	100	0	0	20	100	90	0	0	0
41 → 33 C[c]	100	0	0	100	5	0	100	0	0

[a] Antigens were identified by the indirect immunofluorescent technique. Equal numbers of T and C antigen-containing cells were present at 33, 39, and 41 C. The values were rounded off for the sake of clarity. The standard error of the mean was ± 15%.

[b] The cells were fixed and stained 72 h after infection at 33 C and after 48 h at 39 or 41 C.

[c] The cells were incubated at 41 C for 48 h, shifted to 33 C, and incubated a subsequent 24 h before fixation and staining.

described an 80,000 dalton "Y" protein present inconsistently in whole-cell extracts of CV-1 cells (1). Walter, Roblin, and Dulbecco found 60,000 and 70,000 dalton proteins in nuclei extracted from Vero cells (25). Kiehn did not report any similar proteins in nuclear extracts of BSC-1 cells but did find a 76,000 dalton protein in the cytosol of infected cells (12). Del Villano and Defendi identified a 70,000 dalton protein in extracts of cells transformed or acutely infected by SV40 by using specific immunosorbents reacting with T antigen (4). A comparison of these high molecular weight proteins will require further purification and characterization by immunological and chemical techniques.

The 72,000 dalton protein which we have described could be a specifically induced cell protein, a precursor of capsid protein, or a noncapsid viral protein which accumulates in the absence of normal late viral functions. The high molecular weight protein is probably not an uncleaved precursor of capsid proteins because it is relatively stable after a shift to the permissive temperature, it reacts poorly with antiserum prepared against SV40 capsid proteins, and VP1, VP2, and VP3 are synthesized at a normal rate in infection by tsB11.

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