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Genetic Analysis of Adenovirus Type 2

III. Temperature Sensitivity of Processing of Viral Proteins

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Using sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis of [^{35}S]methionine-labeled adenovirus type 2-infected KB cell extracts, a total of 23 virus-induced polypeptides was detected. This technique was applied to the analysis of the temperature-sensitive mutant, ts 1, which has previously been shown to be defective in a late function. By means of pulse-chase experiments, ts 1 was shown to be defective in the processing of the precursor polypeptide (Pre VII) to the major core protein VII. Two other putative precursor polypeptides, Va (27K) and Vb (24K), were also not processed. Thus, the ts 1 mutation blocked the appearance of six post-translational cleavage products, i.e., polypeptides VI, VII, VIII, X, XI, and XII. All of these polypeptides are virion components. Processing was temperature sensitive in a shift-up experiment, whereas it was normal in a shift-down experiment. The kinetics of the temperature-shift experiments suggested that infectious virus could be recovered if enough time is provided for processing to take place. Processing was not inhibited by cycloheximide. The analysis of purified virus particles and empty shells (TCs) revealed the presence of the precursor and putative precursor polypeptides Pre-VII, Va and Vb, instead of their cleavage products, in both types of particles. Based on these results we propose that the ts 1 gene codes for or regulates an endoprotease which is responsible for the completion of the last step in virus maturation, that is, the conversion of "young virions" into mature infectious virions by a series of maturation cleavages.

The biochemical analysis of adenovirus-induced events using conditional lethal mutants has been the subject of several recent communications (8, 11, 18, 19). We have previously reported the isolation and preliminary characterization of adenovirus type 2 (Ad2) temperature-sensitive (ts) mutants (2, 21). In an effort to study the nature of the ts mutations we began the analysis of one particular mutant, ts 1. At the restrictive temperature, this mutant produces a normal yield of noninfectious physical particles (21). The infectious virus produced at the permissive temperature exhibits increased thermolability (21). Consequently the ts 1 mutation affects the structural proteins. The present communication will demonstrate that the ts 1 mutation blocks the post-translational processing of several virus-induced precursor proteins (1, 3, 9).

(A report of this investigation was presented at the annual meeting of the American Society for Microbiology, New York City, 1975).

MATERIALS AND METHODS

Cells and virus. KB cells (obtained from Flow Laboratories, Rockville, Md.) were grown in Dulbecco modified minimal essential medium prepared in the laboratory, and supplemented with 10% heat inactivated calf serum, penicillin (100 U/ml), and streptomycin (100 μg/ml). During the course of experiments the calf serum was reduced to 2.5% and 0.4 mM arginine was also added. Experiments were performed in 5-cm Falcon plastic petri dishes or more often in 24-well (16-mm diameter/well) Linbro plates (Bellco Glass Inc., Vineland, N.J.). The wild-type (WT) Ad2 was the parental strain of the ts 1 used in all experiments. ts 1 is identical to the ts 241 of the first paper in this series (2). All experiments were performed with a single stock of third passage ts 1 virus (at 33 C) with a titer of 1.9×10^{10} plaque-forming units per ml. The infectivity ratio was determined by a fourth passage of a sample at 33 and 39 C and titrating the yields at 33 C: we thus obtained a 33 C/39 C infectivity ratio of 2×10^2.

Virus was purified by the following procedure. Cells were freeze-thawed three times, sonicated for 20 s, and clarified by low-speed centrifugation, and the supernatant was extracted twice with trichlorotrifluoroethane (Freon 113, Dupont of Canada, Ltd.) as described previously (7). This material was gently pipetted on top of a preformed CsCl gradient (1.2 to 1.5 g/ml) and centrifuged for 1 h at 27,000 rpm in an SB283 rotor of the International B60 Ultracentrifuge.

Fractions were collected from the bottom of the tube, the density was determined via the refractive index, and the separated mature virions and top components (TCs) were diluted with buffer (0.05 M Tris-hydrochloride, pH 8.1) and separately centrifuged to equilibrium in CsCl (1.4 g/ml) in the same rotor at 24,000 rpm overnight. The viral material was pressure dialyzed and concentrated by ultrafiltration (Amicon, Lexington, Mass.).

Labeling of cells and virus with [^{35}S]methionine. Confluent monolayers of KB cells in Linbro plates were infected by the addition of 5 to 10 plaque-forming units of virus per cell in 25 μl of Tris-buffered saline (TBS). Mock infections consisted of 25 μl of TBS only. The cultures were gently agitated at 15-min intervals for 1 h at 33 C, then 1 ml of fresh medium was added and incubation was continued at 33 C or 39 C. Immediately before labeling the medium was replaced with 0.5 ml of Dulbecco modified minimal essential medium containing 1/20 the normal concentration of methionine, and 10 μCi of [^{35}S]methionine per ml (about 250 Ci/mmol; Amersham-Searle). Unless otherwise stated the cells were labeled for 2 h at 33 C and 1 h at 39 C. At the end of the pulse the cells were rinsed once with TBS and the culture was either lysed immediately or chased by the addition of complete medium containing a 100-fold excess of cold methionine (3 mg/ml). At the end of the pulse or chase, the cells were collected into a 0.1-ml sodium dodecyl sulfate (SDS) sample solution (0.05 M Tris, pH 6.8; 1% SDS, Matheson-Coleman; 10% glycerol; 0.1% mercaptoethanol; 0.001% phenol red), boiled for 1 min, and cooled, and either equal volume or equal radioactivity was applied to the gels. [^{35}S]methionine-labeled virus was prepared as follows. KB cell monolayers in 5-cm plastic petri dishes were infected as above, but the volumes were adjusted to fourfold. Approximately 6 to 8 h postinfection, [^{35}S]methionine was added to a final concentration of 10 μCi/ml. Labeling was repeated at intervals two to three times. At the end of the growth cycle, i.e., 48 h at 39 C and 72 h at 33 C, the virus was purified as described above. Contrary to others (9), in our experience virus would not grow in medium containing 1/20 the normal concentration of methionine. Addition of complete medium during the last third of the growth cycle, however, was found to restore a normal yield. For this reason, marker virus was routinely prepared in complete medium.

SDS-polyacrylamide gel electrophoresis. The polyacrylamide gel system was basically that described by Maizel (12). The SDS-disc system was used with the Hoeffer slab-gel apparatus (Hoeffer Scientific, San Francisco). The gels (0.75 mm thick, 10.5 cm high) consisted of a 12.5% resolving gel and a 5% stacking gel with an acrylamide-bisacrylamide ratio of 30:0.8. The gels were run at 30 mA/slab, stained with Coomassie brilliant blue, dried in vacuo, and autoradiographed with Kodak RP-oxomat medical X-ray film.

RESULTS

Time-course of virus-induced protein synthesis. In a previous communication (21) we showed that ts 1 produces virus particles at the nonpermissive temperature with an efficiency equal to WT. Since these ts 1 particles are not infectious we compared the pattern of ts 1 and WT virus-induced protein synthesis. KB cells were infected with 5 infected-cell units (ICU)/cell of ts 1 or WT and incubated at 33 or 39 C. At various times after infection the cultures were pulse labeled with [^{35}S]methionine for 1 h, and the cells were collected in SDS-mercaptoethanol sample solution and analyzed by electrophoresis on polyacrylamide gels (Fig. 1). The ordered pattern of the increase of synthesis of viral proteins and the concomitant decrease in the synthesis of cellular proteins was consistent with previous reports (1, 20). All the late virus-induced polypeptides appeared between 9 to 12 h after infection at 39 C and between 18 to 22 h after infection at 33 C. Although hexon polypeptide was sometimes detectable somewhat earlier than the rest, this may be explained by its greater ease of detection. The 72K polypeptide was found to be the only early protein detectable in these gels. It became visible 3 and 9 h postinfection, at 39 and 33 C, respectively. However no qualitative or significant quantitative differences in infected cell polypeptide synthesis were detected at either incubation temperature between ts 1 and WT (results not shown).

Comparison of ts 1 and WT by pulse-chase experiments. It has been shown that several adenovirus proteins are derived by cleavage of precursor proteins (1, 3, 9). Preliminary experiments with long labeling periods revealed differences in the polypeptide patterns of ts 1-infected cells at the nonpermissive temperature. To test the hypothesis that ts 1 is defective in the processing of precursor proteins, a pulse-chase experiment was performed. KB cells infected with ts 1 and WT were labeled with [^{35}S]methionine 20 h (39 C) or 40 h (33 C) after infection for 2 h. The radioactive medium was then replaced with medium containing 100-fold excess methionine, and incubation was continued for various lengths of time. The cells were harvested and analyzed on SDS-polyacrylamide gels (Fig. 2). Once more the pulse-labeled samples of ts 1 and WT were identical; however, several differences may be observed during the chase. First, a heretofore undescribed infected cell-specific band of approximately 95,000 daltons (95K) disappeared during the chase. Second, a new, diffuse infected cell-specific band of 73,000 daltons (73K) appeared after 4 h of chase. There appeared to be no relationship between these polypeptides, as the material

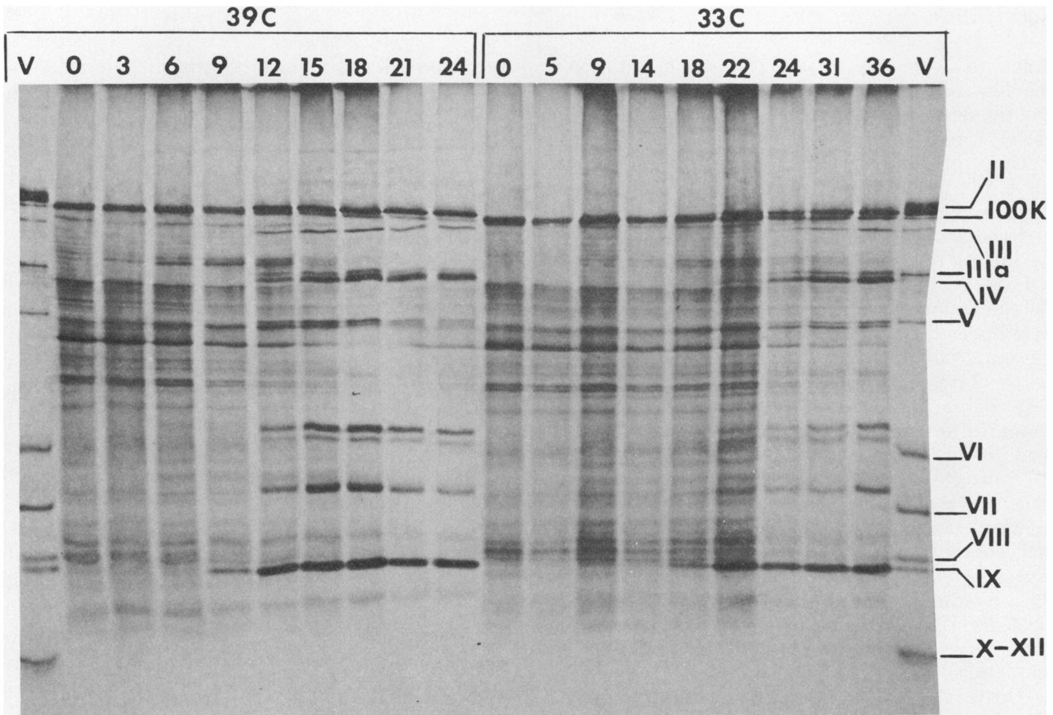

FIG. 1. *SDS-polyacrylamide gel autoradiogram of ts 1-infected KB cells at 33 or 39 C, pulse-labeled at different times after infection. Cells were labeled for 1 h (39 C) or 1.5 h (33 C) with [^{35}S]methionine, then solubilized in SDS sample solution, and electrophoresed as described. The sample order is (from left to right): ts 1-infected at 39 C and pulse-labeled at 3, 6, 9, 12, 15, 18, 21, and 24 h postinfection; V, purified WT grown and labeled at 39 C; ts 1-infected at 33 C and pulse-labeled at 5, 9, 14, 18, 22, 24, 31 and 36 h postinfection; V, purified WT grown and labeled at 33 C.*

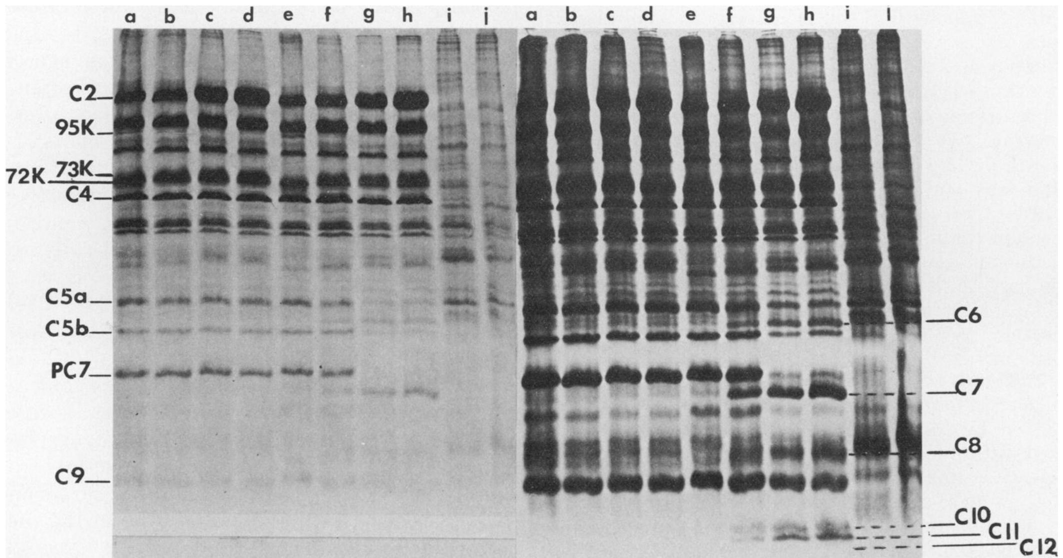

FIG. 2. *SDS-polyacrylamide gel autoradiogram of ts 1- and WT-infected KB cells at 39 C, labeled for 2 h, and then chased in nonradioactive medium. The details of experimental procedure are as described. To show both the high- and low-molecular-weight regions of the gel, a short and long exposure of the same gel were made. The sample order is: (a to d) ts 1-infected cells; (e to h) WT-infected cells; (a and e) 2-h pulse; (b and f) 4-h chase; (c and g) 19-h chase; (d and h) 26-h chase; (i) mock-infected cells pulse-labeled for 2 h, and (j) chased for 26 h. The designations increase in order of increasing mobility with the exception of the reversed order seen for C5b and C6, a phenomenon encountered only on this gel and observed before by Walter and Maizel (20).*

which appeared in the 73K band seemed far in excess of that which disappeared from the 95K band. The 73K polypeptide may represent a post-translational modification of the 72K single-stranded DNA binding protein (11, 19). Third, and this is the only difference between ts 1 and WT, C5a, C5b, and PC7 polypeptides failed to diminish and C6, C7, C8, C10, C11, and C12 polypeptides failed to appear during the chase in ts 1-infected cells. The six cleavage products are virion components and have been described previously (1, 9, 10, 15). A precursor-product relationship has only been documented between PC7 (PVII) and C7 (VII) (1, 9, 20). The present pulse and chase experiments were consistent with a previously suggested tentative precursor-product relationship between C5a and C6 (or VI), and between C5b and C8 (or VIII) (9, 20). These experiments have been repeated with HEp-2 cells (data not shown) and the same results were obtained. From this experiment it may be concluded that ts 1 is defective in the processing of precursor proteins.

Temperature-shift experiments: polypeptides. Since processing was defective at the nonpermissive temperature, two questions can be raised. (i) Can precursors synthesized at 39 C be chased into cleavage products at 33 C? and (ii) can precursors synthesized at 33 C be chased into cleavage products at 39 C? To answer the first question, KB cells infected with ts 1 and WT at 39 C were pulse labeled with [^{35}S]methionine at 20 h postinfection and chased in medium containing excess cold methionine, at 33 C for 4, 20, and 40 h. The cells were analyzed on SDS-gels as usual (Fig. 3). It is clear that processing is rescued by shifting the infected cells to the permissive temperature. The rate of processing, however, appeared to be slower in ts 1 by about twofold. It is interesting to note that the appearance during the chase of polypeptides C6, C7, C8, and C10–12 seemed to be concomitant.

To answer the second question, infected cells were pulse labeled at 33 C and chased at 33 or 39 C for 20 h. Figure 4 shows the SDS gel autoradiogram. No detectable C6 or C7 polypeptides appeared in the shift-up cultures of ts 1, whereas the controls, consisting of WT and ts 1 chased at 33 C, showed a normal pattern of cleavage. Polypeptide C8 could not be resolved on this gel.

The intracellular site of processing of PC7 has been shown to take place in the nucleus (1). Using a 1-h pulse followed by a 20-h chase we separated nucleus and cytoplasm by means of 1% Triton X-100 (in water), gentle vortexing, and incubation for 15 min at 0 C followed by centrifugation at 2,000 × *g* for 5 min at 4 C (1). SDS gel analysis confirmed the nuclear site of processing. In addition, in ts 1-infected cells at the nonpermissive temperature, we observed the nuclear accumulation during the long chase of all the viral proteins and notably the precursor proteins C5a, C5b, PC7. These results lend further support to existing evidence (1, 9, 20) that the precursor proteins are transported to the nucleus and processed there.

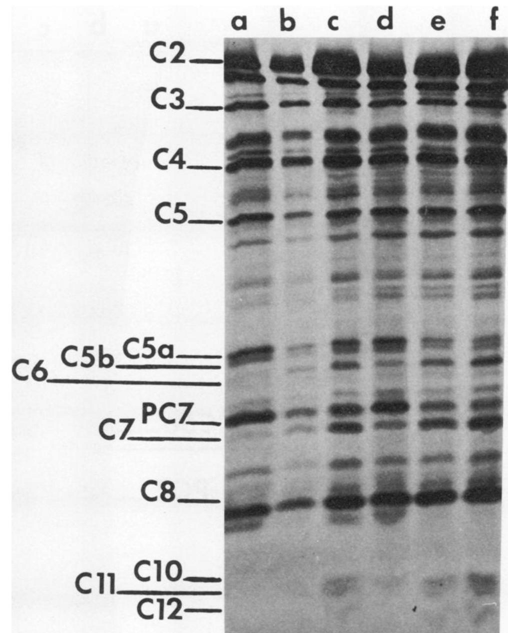

FIG. 3. *Autoradiogram of [^{35}S]polypeptides pulse-labeled at 39 C and chased at 33 C. Cells infected at 39 C with ts 1 or WT were labeled with [^{35}S]methionine for 1 h at 20 h postinfection and chased at 33 C for various times. Approximately the same number of cells were subjected to electrophoresis. (a) ts 1, 4-h chase; (b) ts 1, 20-h chase; (c) ts 1, 40-h chase; (d) WT, 4-h chase; (e) WT, 20-h chase; (f) WT, 40-h chase.*

Temperature-shift experiments: infectious virus. The temperature-shift experiments have shown that, regardless of the temperature at which the precursor polypeptides were synthesized, processing only takes place at the permissive temperature. Therefore, it would be instructive to know the time course of the temperature shift-up and shift-down profiles of infectious virus synthesis. Confluent KB cell cultures in 5-cm petri dishes were infected with 5 to 10 plaque-forming units/cell at 33 C for 1 h. To ensure synchrony of infection the cells were rinsed three times with isotonic TBS, incubated 15 min with Ad2 antiserum, and again rinsed with three changes of TBS. Five milliliters of medium containing 2.5% calf serum

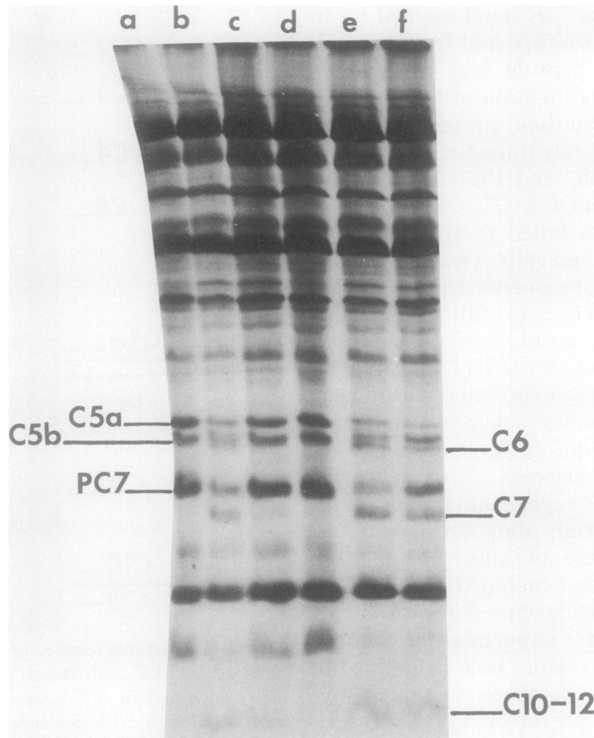

FIG. 4. *Autoradiogram of ^{35}S-labeled polypeptides pulse-labeled at 33 C and chased at 39 C. Cells infected at 33 C with ts 1 or WT were labeled with [^{35}S]methionine for 2 h at 40 h postinfection and chased at 33 or 39 C. (a) ts 1, 2-h pulse at 33 C; (b) ts 1, 20-h chase at 33 C; (c) 20-h chase at 39 C; (d) WT, 2-h pulse at 33 C; (e) WT, 20-h chase at 33 C; (f) WT, 20-h chase at 39 C.*

and 0.4 mM arginine was added to the cultures and incubated for various lengths of time at 33 or 39 C. To obtain growth curves at the permissive and nonpermissive temperatures, cultures were frozen after various times of incubation at 33 or 39 C, respectively. For the temperature-shift experiments, at various times after infection, duplicate cultures were shifted from the permissive to the nonpermissive temperature, or vice-versa. All temperature-shift cultures were harvested at 72 h postinfection by collecting both cells and medium. Crude cell lysates were titrated by plaque formation. The experiment was repeated four times and the mean titers were used to construct the profiles shown in Fig. 5. A comparison of the growth curves at 33 and 39 C shows that ts 1 grew to a maximum titer at 39 C which is 0.1% of that at 33 C. The temperature shift-up profile reveals a complex, reproducible pattern, showing depression of the final virus yield below the level that would have been obtained without shifting early in the infectious cycle. The reasons for this novel inhibitory effect are not known. However, by 30 h postinfection, the shift to the nonpermissive temperature has a diminishing inhibitory effect, indicating that the ts 1 gene defect is expressed primarily prior to 40 h postinfection at 33 C.

The temperature shift-down profile shows that 20 to 30 h were required at the permissive temperature to avoid the mutant effect. This is in agreement with the above results showing that processing may be a rate-limiting step in adenovirus maturation. It should be noted that, with the exception of the peculiar initial depression in the shift-up experiment, the shift-down profile and the 39 C growth curve meet exactly at the yield level that is characteristic of this mutant.

Cycloheximide fails to inhibit processing. Since processing appears to be a relatively slow process, a question arose: is protein synthesis required for processing of the precursor proteins? KB cells infected with WT virus at 39 C were labeled for 1 h at 20 h postinfection and chased in the presence or absence of 100 μg of cycloheximide per ml for 24 h. Under these conditions the total incorporation of [^{35}S]methionine into the acid-precipitable pool was reduced by 80%, as measured in a parallel experi-

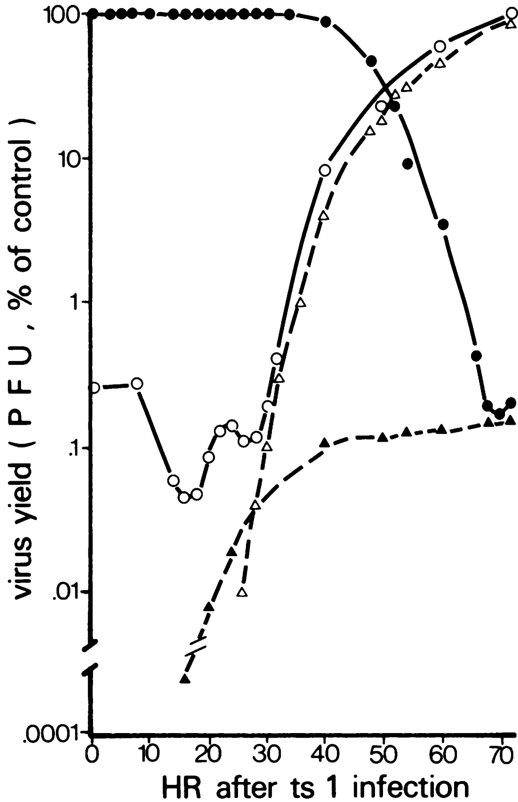

FIG. 5. *Temperature shift-up and shift-down experiments of ts 1. Replicate cultures of synchronously infected KB cells (see text for details) were incubated at 33 or 39 C for various lengths of time and then (a) shifted to the other temperature for the temperature-shift experiments, or (b) frozen and harvested to determine the growth curves at both temperatures. The temperature shifted cultures were always incubated a total of 72 h. All yields were titrated by plaque formation at 33 C and expressed as a percentage of the control, or 72 h yield at 33 C (ordinate). The abscissa gives the hour after ts 1 infection when the culture was frozen or shifted. Symbols: ○, shift-up experiment; ●, shift-down experiment; △, 33 C growth curve; ▲, 39 C growth curve.*

ment. The SDS-polyacrylamide gel profiles (Fig. 6) of the infected cell polypeptides remained unchanged when the chase was performed in the presence of cycloheximide. It may be concluded that new protein synthesis does not appear to be required for processing to continue at the usual rate.

The polypeptide composition of ts 1 virions and top components (TCs). We have previously shown by physical methods that ts 1 produces a normal yield of noninfectious physical particles at the nonpermissive temperature (21). This has been confirmed by electron microscopy, as shown in Fig. 7. The above results indicate that several virion proteins are not produced at the nonpermissive temperature. In the following experiments, we wish to examine the polypeptide composition of mutant virus particles to test the prediction that these particles do not contain polypeptides VI, VII, VIII, and X-XI-XII complex.

WT and ts 1 virus was grown at 33 and 39 C in complete medium containing 10 μCi of [^{35}S]methionine per ml. Virions and TCs were purified by velocity-equilibrium sedimentation for 1 h followed by an overnight equilibrium sedimentation in CsCl. The visible viral band and the TCs bands were collected separately, dialyzed, and prepared for electrophoresis on SDS gels. The results (Fig. 8) show several interesting features. ts 1 virions, grown at the permissive temperature, also contain, in addition to the normal set of polypeptides, polypeptides Va, Vb, and Pre-VII. ts 1 virions grown at the nonpermissive temperature contain only Va, Vb, and Pre-VII, whereas the cleavage

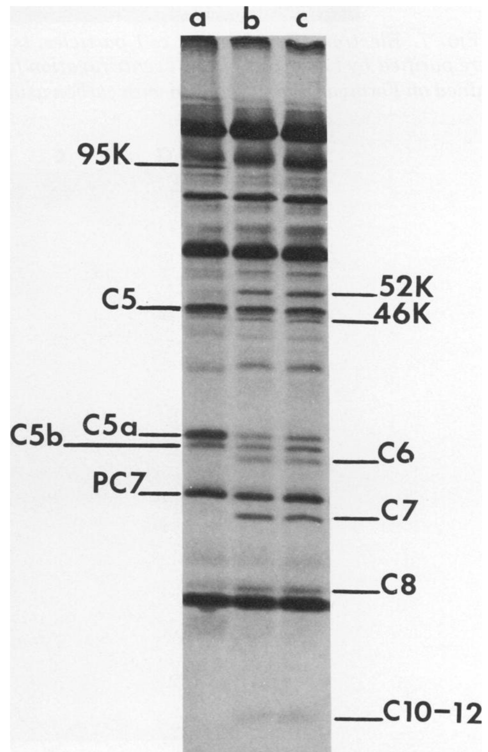

FIG. 6. *The effect of cycloheximide on the processing of ^{35}S-labeled peptides. KB cells infected with WT at 39 C were labeled for 1 h and chased in the presence or absence of cycloheximide (100 μg/ml) for 24 h: (a) 1-h pulse at 39 C; (b) 24-h chase; (c) 24-h chase in the presence of 100 μg of cycloheximide per ml.*

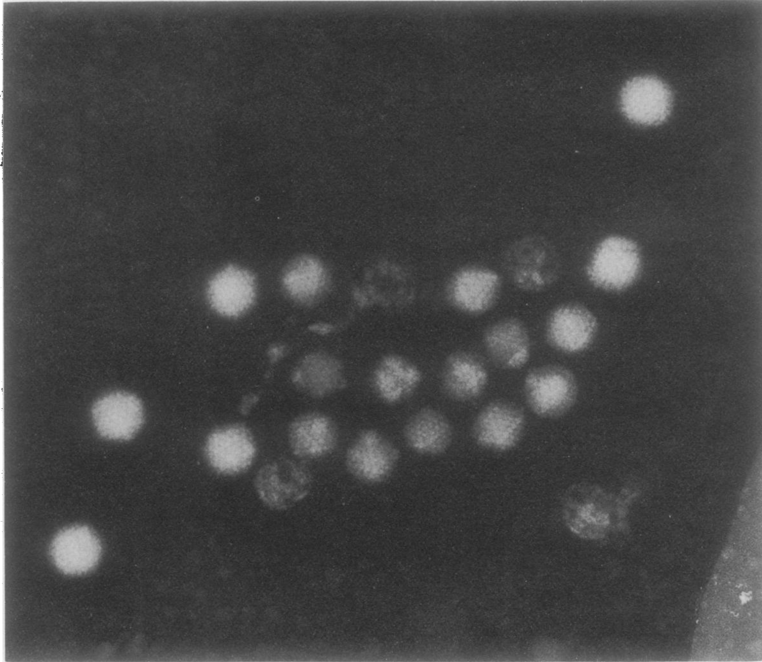

FIG. 7. *Electron micrograph of ts 1 particles. ts 1 was grown at 39 C and the virions banding at 1.34 g/cm³ were purified by two cycles of CsCl centrifugation followed by dialysis as described. The virions were negatively stained on Formvar grids stabilized with carbon using 2% phosphotungstic acid, pH 7. ×90,000.*

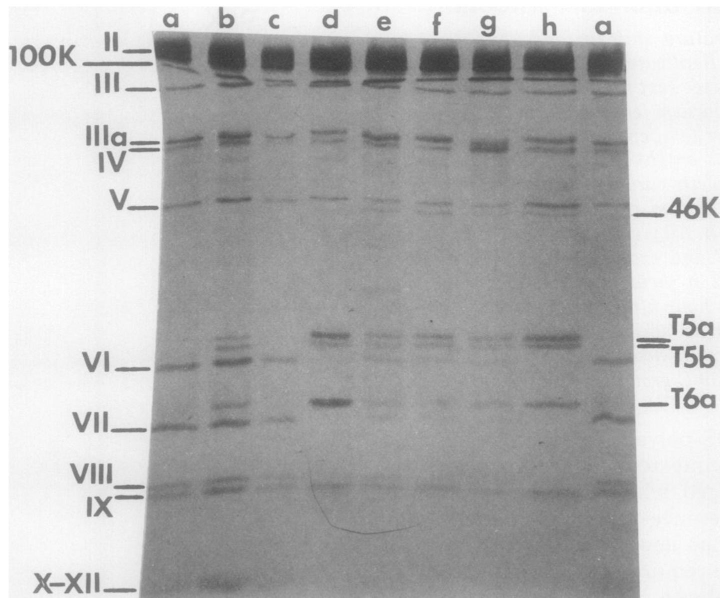

FIG. 8. *Comparison of ³⁵S-labeled polypeptides of ts 1 and WT in virions and TCs. Virus grown in the continuous presence of [³⁵S]methionine was purified by two cycles of centrifugation in CsCl and the major virus band at a density of 1.34 g/ml, and the TCs in the density region of 1.30 g/ml were collected separately for electrophoresis. Approximately equal amounts of radioactivity per sample were used. (a) WT virions at 33 C; (b) ts 1 virions at 33 C; (c) WT virions at 39 C; (d) ts 1 virions at 39 C; (e) WT TCs at 33 C; (f) ts 1 TCs at 33 C; (g) WT TCs at 39 C; (h) ts 1 TCs at 39 C.*

products VI, VII, VIII, and X-XI-XII are completely absent. Therefore, these results are consistent with the conclusions based on the analysis of infected cell polypeptides. Furthermore, they demonstrate that (i) the missing polypeptides are not required for the formation of physical particles; (ii) the presumed precursor polypeptides (Va, Vb, Pre-VII) may remain in a stable association with the mature (DNA containing) virions; (iii) the packaging of DNA into virions may take place in the absence of processing; and (iv) that all these changes result in noninfectious virions.

The TCs of ts 1 and WT are indistinguishable at 33 C but, as expected, at 39 C the ts 1 TCs are identical to the ts 1 virions. The significance of the 46K band visible in all the TCs is unknown at present. It may be concluded that the ts 1 virions represent an accumulating population of intermediate particles in the course of maturation of adenovirus.

DISCUSSION

We have compared the synthesis of virus-specific polypeptides of a ts mutant, ts 1, and the parental WT adenovirus during the late stages of replication in KB cells. At least 15 polypeptides specific for infected cells become visible during a 1-h labeling period with [^{35}S]-methionine (Table 1). They become distinguishable within a background of cellular polypeptide bands between 9 to 12 h postinfection at the nonpermissive temperature, and between 18 to 22 h at the permissive temperature. Under conditions where all the cells are infected, the increasing rate of viral protein synthesis is paralleled by a decreasing rate of host protein synthesis. The approximately halved time course of synthesis at 39 C appears to be maintained throughout the infection cycle. These experiments failed to show any differences between ts 1 and WT.

Short labeling periods followed by relatively

TABLE 1. *Ad2-induced proteins*

Band designation[a]	Molecular weight[b]	Appears during:		Quantity during chase[c]	Relationship to virion
		Pulse	Chase		
II	120,000	+		I	Hexon
IIa (100K)	100,000	+		U	
95K	95,000[d]	+		D	
III	85,000	+		U	Penton base
(72K)	72,000	+		U	DNA binding protein (19)
IIIa	66,000	+		U	Peripentonal area (5)
IV	62,000	+		U	Fiber
IVa (IVa1)	60,000		+	I	Virion component
IVb (IVa2)	56,000		+	I	Virion component
(52K)	52,000[d]		+	I	
V	48,500	+		U	Core
46K	46,000[d]	+		I	DNA binding protein (19)
Va (27K)	26,000[d]	+		D	Presumed precursor to VI (20)
Vb (26K)	24,000[d]	+		D	Presumed precursor to VIII (20)
VI	22,000[d]		+	I	Ninemer (5)
VIa (PVII)	20,000	+		D	Precursor to VII (1)
VIb	19,000		+	I	Presumed cleavage intermediate of VIa (9)
VII	18,500		+	I	Major core
VIII	13,000		+	I	Ninemer (5)
IX	12,000	+		U	Ninemer (5)
X	6,500		+	I	Virion component
XI	6,000		+	I	Virion component
XII	5,000		+	I	Virion component

[a] As assigned by Maizel et al. (13), Walter and Maizel (20), Ishibashi and Maizel (9), Everitt et al. (4, 5), and Anderson et al. (1) (in parentheses) or for nonvirion adenovirus-induced components, expressed as a number based on the apparent molecular weight in 12.5% SDS-polyacrylamide gels. All of the components have been found by autoradiography in [^{35}S]methionine-labeled cells.

[b] As determined by references.

[c] I, Increases; D, decreases; U, unchanged; as judged from autoradiograms.

[d] Determined from the component's mobility in 12.5% SDS-gels using purified [^{35}S]methionine or ^{125}I-labeled virions for comparison and protein markers, bovine serum albumin (68,000), ovalbumin (45,000), chymotrypsinogen A (25,000), ribonuclease A (13,700), cytochrome *c* (12,400), bacitracin (1,450).

long chase periods revealed seven additional infected cell-specific polypeptides. Six of these failed to appear in ts 1-infected cells at the nonpermissive temperature. The present results are in agreement with previous work that these polypeptides are virion components that are cleavage products of precursor polypeptides (1, 3, 9, 20). The precursor-product relationship has been documented between VIa (Pre-VII) and VII, and inferred from kinetic data between Va and VI and between Vb and VIII (9, 20). Pulse-chase experiments at 39 C with ts 1 showed that the putative precursors do not diminish during the chase as is the case with the WT or the mutant at the permissive temperature. This concomitant accumulation of the putative precursors and the failure of the appearance of the cleavage products observed in ts 1 strengthens existing evidence of a precursor-product relationship between Va-VI, Vb-VIII, and VIa-VII, respectively.

Endoproteolytic cleavage of Va (26K) into VI (22K) and Vb (24K) into VIII (13K) would give rise to a 4,000-dalton and an 11,000-dalton polypeptide fragment, respectively. The 4,000-dalton fragment could be a candidate for one or more of the X-XII complex. In our experience these small polypeptides could often not be resolved into more than one band, thus calling into question their authenticity. In any event the appearance of these polypeptide species of approximately 4,000 to 6,000 daltons is under the control of ts 1 gene and thereby possibly related to Va. Such a relationship between X-XII and Vb is unlikely as the cleavage fragment (11,000 daltons) of Vb is far too large. Since this 11K cleavage fragment would comigrate with IX, the present techniques do not allow its observation. Further cleavage of the 11K fragment into two polypeptides cannot be excluded by the present results.

The maturation of adenovirus particles through the intermediate TCs and "young virions" in that order has been well demonstrated (9, 17). The present results strengthen previous evidence that the Ad 2 DNA can be packaged into the TCs without requiring processing of the precursor proteins. This and the work of others has demonstrated that these precursor proteins (Va, Vb, Pre-VII) are incorporated into the TCs (9), which then become young virions after DNA is added, processing taking place subsequently thereby giving rise to the mature virions. DNA is packaged fairly rapidly since virtually no intermediates between empty shells and mature virions are detectable.

The ts 1 physical particles formed at 39 C are not infectious (21). The defect is subsequent to absorption, since this takes place normally (unpublished data). As purified adenovirus DNA is infectious (6, 14) none of the structural proteins are apparently required for the establishment of infection. This would suggest that the lack of infectivity of the 39 C ts 1 particles may be due to a failure to uncoat. Whether such a failure to uncoat is due to the absence of polypeptides VIII and the X-XII complex, or the presence in the particles of the precursor polypeptides Va, Vb, and Pre-VII, is a problem that needs to be investigated. In particular, the presence of Pre-VII in the mature, DNA-containing particles offers the possibility of testing the idea that VII may be involved in joining the two ends of the adenovirus DNA molecule (16).

What is the nature of the defect in the ts 1 mutant? First, several genetic properties of this mutant suggest that it carries a single genetic lesion, namely (i) the reversion frequency is approximately 10^{-4}, (ii) ts 1 complements with mutants in 12 other complementation groups, (iii) ts 1 recombines with members of other complementation groups to produce WT recombinants (2). Second, the present results show that the processing of three precursor polypeptides is arrested by the ts 1 mutation. The ts lesion therefore is a property not of the substrate but of a cleavage enzyme or its synthesis. At least two alternatives are possible: (i) the ts 1 gene product is a virus-coded missense endoprotease, or (ii) a hitherto undetected missense virus protein either induces the synthesis of a cellular endoprotease or is a necessary co-factor to the cellular enzyme for the cleavage of the viral proteins. We are currently investigating these and other aspects of the ts 1 phenotype.

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