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Temperature-Sensitive BC Mutants of Simian Virus 40: Block in Virion Assembly and Accumulation of Capsid-Chromatin Complexes

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We examined the morphology, protein composition, and stability of the nucleoprotein complexes assembled in cells infected with simian virus 40 mutants belonging to the BC complementation group (*ts*BC11, *ts*BC208, *ts*BC214, *ts*B216, *ts*BC217, *ts*BC248, *ts*BC223, and *ts*BC274). We found that the 220S virions were not assembled in tsBC-infected cells under restrictive conditions. This block in assembly resulted in the accumulation of 75S chromatin in tsBC11-infected cells, as previously observed by Garber et al. (E. A. Garber, M. M. Seidman, and A. J. Levine, *Virology* **107**:389–401, 1980). In cells infected with any other mutant listed above, the block in assembly resulted in the accumulation of 75S chromatin as well as nucleoprotein complexes sedimenting from 90 to 140S. Biochemical analysis revealed that these latter complexes contained the capsid proteins in addition to simian virus 40 DNA and the cellular core histones. Electron microscopic analysis clearly showed the association of the capsid proteins with the viral chromatin. Our results suggest that these proteins interact with simian virus 40 chromatin in the course of virion maturation and may thus play an active role in controlling simian virus 40 functions.

Mature simian virus 40 (SV40) contains closed circular, double-stranded DNA and proteins consisting of the major capsid protein (VP1), two minor capsid proteins (VP2 and VP3), the cellular core histones (H2A, H2B, H3, and H4), and a topoisomerase activity (2, 3). The viral genome is packaged within the icosahedral shell in a nucleoprotein structure called the SV40 chromatin or chromosome (15, 30).

Investigations of virion maturation have established that SV40 chromatin (75S) is produced after the completion of viral DNA replication. During this process, the cellular core histones fold the newly replicated DNA into nucleosomes (1, 9, 11–14, 17, 18, 26), the fundamental units of DNA packaging in higher cells (reviewed in reference 22). The chromatin thus produced can reenter the replicative pathway, be used as a template for the selective transcription of the SV40 early or late genes, or enter the pathway leading to virion production (30). Pulse-chase experiments have revealed that a fraction of chromatin appears in the 200S previrions immediately after its synthesis (1, 9, 11–14). The previrions are then converted to the 220S salt-labile or immature virions, which mature with time and yield stable virions (9, 11). These results, together with the finding that shells lacking chromatin do not accumulate in the infected cells, lead to the hypothesis that SV40 assembly proceeds via the direct addition of the capsid proteins to chromatin (9, 11, 14). Despite these recent advances, we know little about the mechanisms governing the initiation, or nucleation, of shell assembly on SV40 chromatin or about the molecular interactions favoring shell polymerization.

To understand the SV40 morphogenetic pathway better, we have been studying the nucleoproteins produced in cells infected with the SV40 temperature-sensitive mutants (designated *ts*) in assembly (3, 3a, 5). These mutants have been mapped to the gene coding for the major capsid protein VP1 (20) and have been separated into three groups—*ts*B, *ts*C, and *ts*BC—by complementation analysis (7, 8, 20, 23, 28). The *ts*B and *ts*C mutants complement each other, but not *ts*BC, under restrictive conditions (7, 8, 20). Using marker rescue analysis, Lai and Nathans (20) demonstrated that the *ts*B/C mutations show clustering: eight of nine *ts*B mutations map to the VP1 amino terminus; the *ts*C mutations are primarily localized in the central region; and seven of eight BC mutations occur in the carboxyl end. Lai and Nathans thus proposed that these mutations may affect various functional domains of the VP1 protein, *ts*B and *ts*C complementing one another by protein-protein interactions (20).

In agreement with the Lai and Nathans hypothesis (20), we find that the *ts*C and *ts*B mutants yield distinct types of nucleoprotein complexes under restrictive conditions (3, 3a, 5). The *ts*C mutants accumulate only chromatin (3). In cells infected with the *ts*B mutants, the capsid proteins interact with chromatin, yielding 100 to 160S nucleoproteins (3, 3a, 5). When examined by electron microscopy, these complexes appear as semiassembled virions and readily dissociate when exposed to high-ionic conditions (3, 3a, 5).

There was no correlation between the *ts*BC mutations and the type of nucleoproteins assembled at 40°C when two mutants of this group were studied. Garber et al. found that in cells infected with *ts*BC11, assembly does not proceed further than 75S chromatin (14). We observed that in *ts*BC216-infected cells, 75S chromatin associated with the capsid proteins to form heterogeneously sedimenting nucleoprotein complexes (HSN) (3a). These HSN consisted of chromatin to which a small cluster of the capsid proteins was attached in the form of a protein knob and were, therefore, morphologically distinguishable for the semiassembled particles formed in *ts*B-infected cells (3a, 5). In addition, although our results (3a) indicate that at 40°C the VP1 protein is stable and is transported to the nuclei of cells infected with *ts*BC216, studies conducted on *ts*BC11 suggest that at this temperature the VP1 protein is not transported and degraded in the cytoplasm (29). This inconsistency led us to reexamine the nucleoprotein complexes formed in cells infected with *ts*BC11 and to investigate the properties of several other mutants of the *ts*BC group by electron microscopy and biochemical methods. These data provided a more detailed

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picture of the components and the stability of nucleoproteins assembled in cells infected with *ts*BC group of SV40 mutants.

MATERIALS AND METHODS

Cell culture and virus. The BSC-40 cells were derived by adapting the BSC-1 line of monkey kidney cells to grow at 40°C, as described (6), and were used for the propagation of virus stocks or for all the experiments. SV40 strain 776 was used as the wild-type virus. We obtained the *ts*BC mutants belonging to the 200 series from R. G. Martin of the National Institutes of Health. *ts*BC11 was obtained from Ann Roman of the Indiana University School of Medicine.

Labeling of DNA and proteins; extraction of nucleoprotein complexes. Confluent BSC-40 cells were infected with the wild-type strain or with an appropriate *ts*BC mutant at a multiplicity of infection of about 50 PFU per cell at 37°C. The infected cells were shifted to the desired temperature 8 h before an overnight labeling with either [^{3}H]thymidine (Schwartz/Mann, 66 Ci/mmol) or [^{3}H]leucine (Amersham Corp., 32 to 58 Ci/mmol). For DNA labeling, each 150-mm culture dish was exposed to 100 μCi of [^{3}H]thymidine in 2 ml of regular medium containing 5% fetal calf serum. For protein labeling, each dish was exposed to 90 μCi of [^{3}H]leucine in 2 ml of leucine-free medium containing 5% dialyzed fetal calf serum.

The nucleoproteins were extracted from cells by mild isolation procedures (1, 3, 3a, 5, 11–14, 17, 18, 26). Briefly, the cells were harvested in cold Hanks balanced salt solution (0.14 M NaCl, 5 mM KCl, 0.4 mM $MgSO_4$, 0.5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.4 mM Na_2HPO_4, 0.4 mM KH_2PO_4, 5 mM D-glucose) and concentrated by low-speed centrifugation (5 min, 3,000 rpm). The pelleted cells were suspended twice in buffer B (10 mM HEPES [*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid], 5 mM KCl, 0.5 mM $MgCl_2$ [pH 7.8]) in the presence of 1 mM phenylmethylsulfonyl fluoride. The SV40 nucleoprotein complexes were extracted from these cells by three alternative methods. Method 1 was used for preparing a total cell extract. The cells were Dounce homogenized (30 strokes) and shaken for 1.5 h at 4°C. The cellular chromatin was then pelleted by centrifugation in a Beckman microfuge, and the supernatant was analyzed by sedimentation at 4°C in 5 to 31% sucrose gradients (made up in 50 mM Tris, pH 7.5) with an SW41 rotor at 40,000 rpm for 1 h. Fractions of 0.4 ml were collected and analyzed for radioactivity by scintillation spectrometry. Method 2 was used for preparing nucleoproteins from the nuclei by following a modification of the Nonidet P-40 (NP-40) procedure (12, 26, 28). The cells were harvested in Hanks solution, concentrated, and equilibrated with buffer B as above. To this, an equal volume of 1% solution of NP-40 made up in buffer B was added to bring the NP-40 final concentration to 0.5%. The nuclei were obtained by centrifugation at 3,000 rpm for 5 min and were suspended in buffer B, Dounce homogenized, and repelleted after gentle shaking for 1.5 h at 4°C. The nuclear extract thus prepared was analyzed by sedimentation as

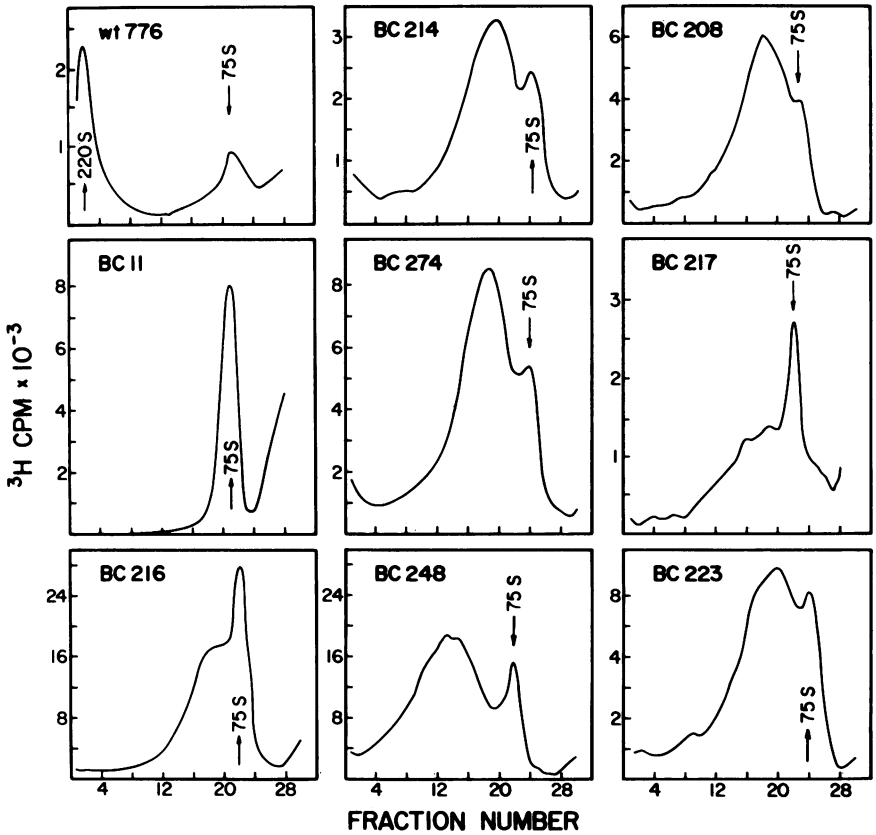

FIG. 1. Velocity gradient analysis of wild-type and *ts*BC nucleoprotein complexes. BSC-40 cells were infected with *wt*776 or with a mutant of the BC group. The infected cells were labeled at 40°C with [^{3}H]thymidine for 12 h before harvest. The nucleoprotein complexes were extracted from cells at 48 h postinfection by method 1 as described in the text and were analyzed in a 5 to 31.5% sucrose gradient. The arrows at 220 and 75S mark the sedimentation positions of SV40 virions and chromatin, respectively.

above. Method 3 was used for preparing a nuclear extract without using NP-40. For this, the cells were preequilibrated with buffer B and then Dounce homogenized 10 times. The nuclei were pelleted by low-speed centrifugation (3,000 rpm, 5 min), and the cytoplasmic fraction was removed. The pellet was suspended in buffer B and shaken gently at 4°C for 1.5 h. The cellular chromatin was removed by centrifugation in a microfuge, and the supernatant containing the nucleoprotein complexes was analyzed by sedimentation in sucrose gradients as described for Method 1.

Protein analysis. The infected cells were labeled with [^{3}H]leucine as described above. The extracted nucleoprotein complexes were fractionated by sedimentation. The DNA-containing fractions were localized by analyzing a sample of each fraction by agarose gel electrophoresis as previously described (2). Appropriate gradient fractions were pooled and concentrated by sedimentation at 45,000 rpm in an SW50.1 rotor at 4°C for 12 to 15 h (10). The pellets were dissolved in a sample buffer, boiled, and analyzed by 16% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (5, 19). The ^{3}H-labeled proteins were then detected by fluorography (1).

Electron microscopy. Sample preparations for electron microscopy were made as described previously (4, 5, 25).

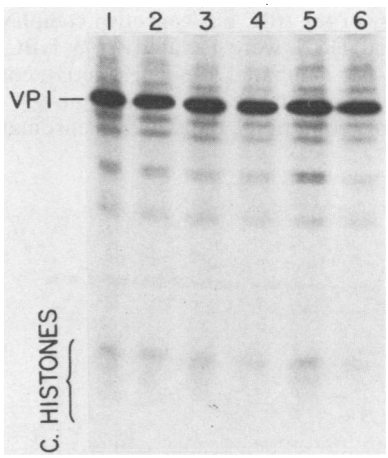

FIG. 2. SDS-PAGE fluorogram of nucleus-extracted *ts*BC216 nucleoprotein complexes. The cells were labeled with [^{3}H]leucine at 40°C. The nucleoprotein complexes were extracted from nuclei prepared by the NP-40 procedure and were fractionated in a sucrose gradient. An equal number of fractions was pooled across the 100 to 220S region of the gradient, concentrated, and analyzed for proteins by SDS-PAGE. The bands were detected by fluorography.

RESULTS

From the numerous temperature-sensitive mutants belonging to the BC complementation group, we chose to study those which were further characterized by Lai and Nathans (20)—namely, *ts*BC11, *ts*BC208, *ts*BC214, *ts*BC216, *ts*BC217, *ts*BC248, *ts*BC274, and *ts*BC223—to reduce the possible effects of secondary mutations on our results. The first mutant, *ts*BC11, was isolated by nitrosoguanidine mutagenesis of the wild-type strain VA45-54 by Tegtmeyer et al., who named the mutant *ts*B11 (27). Lai and Nathans reclassified this mutant as a member of the BC group based on a complementation test by infectious center assay (20). Chou and Martin isolated the other *ts*BC mutants by treating the wild-type SV40 virions (SVS strain, which is the same as strain 776 [20]) with hydroxylamine (7, 8). Lai and Nathans further showed that a number of BC mutants can be rescued with specific DNA restriction fragments from a wild-type virus (20). They thus mapped the mutations in all the BC mutants listed above to the *Hin*-G fragment on the SV40 genome, except *ts*BC223, which was rescued with *Hin*-J (20).

Nucleoprotein complexes isolated from *ts*BC-infected cells. In previous studies, Garber et al. showed that at 40°C, SV40 assembly does not proceed further than the 75S chromatin stage in cells infected with *ts*BC11 (14). We, however, presented evidence that in *ts*BC216-infected cells, the chromatin associated with the capsid proteins to form nucleoproteins sedimenting primarily in the 90 to 160S region of the gradient, with a peak at about 100 to 130S (3a). In the present study, we compared the sedimentation profile of the nucleoprotein complexes extracted from cells infected with the *wt*776 or the *ts*BC mutants listed above. The infected cells were labeled for 12 h with [^{3}H]thymidine at the restrictive temperature (40°C).

As observed previously (1, 3, 3a, 5, 9, 11–14, 17, 18, 24), we obtained two major classes of nucleoprotein complexes, chromatin at 75S and virions at 220S, from cells infected with the wild-type virus (Fig. 1). We found that all the *ts*BC mutants exhibited defects in virion assembly. However, *ts*BC11 was the only mutant which accumulated chromatin (Fig. 1). From cells infected with any other mutant of the BC group, we isolated HSN (90 to 160S) in addition to 75S chromatin (Fig. 1). We therefore conclude that the *ts*BC11 mutant differs from other mutants belonging to the BC group with respect to the types of nucleoproteins assembled at 40°C.

State of the major capsid protein produced in *ts*BC-infected cells. Tegtmeyer et al. (29) analyzed the proteins synthesized in cells infected with *ts*BC11 and observed that the mutationally altered VP1 was not stable at 40°C and thus failed to accumulate in the nucleus. We found that the major capsid protein synthesized at 40°C in the *ts*BC216-infected cells appeared stable (3a). To extend this result and to compare it with that reported for *ts*BC11, we examined the state of the VP1 protein obtained from the nuclei of cells harboring *ts*BC216 by following a modified form of the extraction procedure described for *ts*BC11 (29). Briefly, the *ts*BC216-infected cells were labeled with [^{3}H]leucine at 40°C and harvested at 48 h postinfection. The nuclear extract was prepared by method 2 as described above and was fractionated by sedimentation in a sucrose gradient. We followed the sedimentation profile of proteins across the gradient by SDS-PAGE. The fluorogram of this gel revealed that a stable VP1 protein was present in the nuclear extract and sedimented primarily in the 90 to 160S region of the gradient (Fig. 2). This result indicates that the *ts*BC216 VP1 is not only stable at 40°C but is also transported to the nuclei, where it associates with chromatin to form HSN.

We further examined the state of VP1 protein synthesized in cells infected with two other mutants of the BC group, *ts*BC248 and *ts*BC223. These mutants were selected for further study because the *ts*BC248 mutation occurs in the same DNA fragment as the *ts*BC11 and *ts*BC216 mutations (*Hin*-G), whereas the BC223 mutation is located in the *Hin*-J fragment (20). For this analysis, cellular extracts were prepared from the infected cells labeled at 40°C, fractionated on sucrose gradients, and examined by SDS-PAGE. The results of the analysis revealed that the VP1 protein produced in cells infected with *ts*BC248 or *ts*BC223 was also stable and cosedimented with HSN, as did the *ts*BC216 VP1 (Fig. 3).

Morphology of the *ts*BC nucleoprotein complexes. The 75S chromatin and HSN were isolated from *ts*BC216-infected cells which were incubated under restrictive conditions and examined by electron microscopy. Figure 4 shows that under low ionic conditions, the *ts*BC216 chromatin exhibited the beads-on-a-string structure like that of the chromatin isolated from wild type-infected cells (15). Figure 4b displays the structure of representative nucleoprotein complexes which sediment from 90 to 120S. We found that 90% or more of the molecules appeared in the micrographs as SV40 chromatin associated with a protein cluster. The diameter of this cluster was about 20 to 25 nm. We observed similar structures when we examined the HSN obtained from *ts*BC223- and *ts*BC248-infected cells (data not presented). Based on the SDS-PAGE studies described above, it is reasonable to assume that the protein cluster attached to chromatin is composed of the viral capsid proteins.

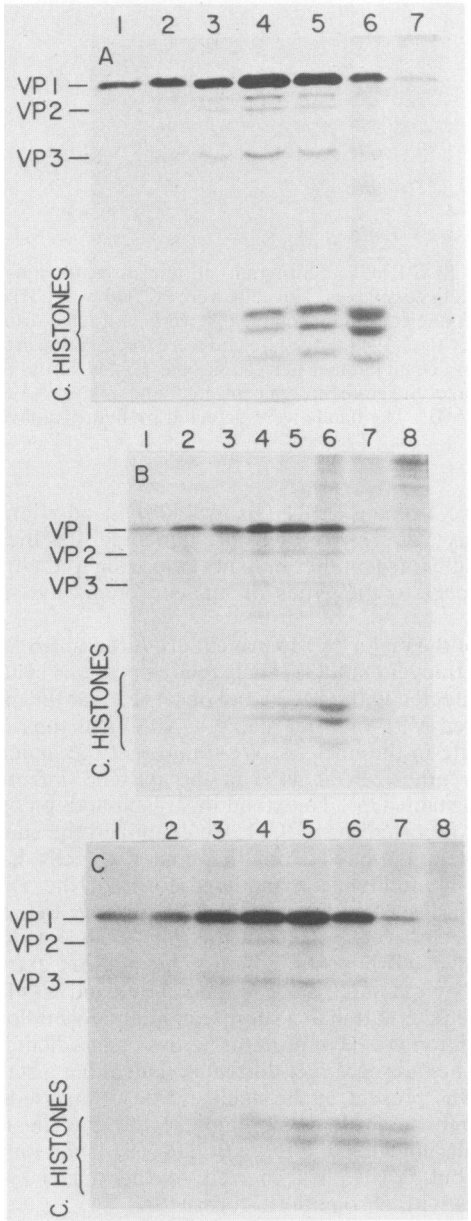

FIG. 3. SDS-PAGE analysis of cellular extract obtained from cells infected with *ts*BC216 (A), *ts*BC248 (B), and *ts*BC223 (C). The infected cells were labeled with [^{3}H]leucine at 40°C. A cellular extract was prepared and fractionated on sucrose gradients as described in the legend to Fig. 1. The appropriate fractions were pooled, concentrated, and analyzed by SDS-PAGE. (A) Lanes 1 to 7 show the proteins coisolating in the 210 to 195S, 185 to 170S, 160 to 145S, 135 to 120S, 110 to 100S, 80 to 60S, and 50 to 30S gradient regions, respectively. (B) Lanes 1 to 8 show the proteins coisolating in the 220 to 200S, 190 to 175S, 160 to 145S, 135 to 120S, 110 to 95S, 85 to 75S, 63 to 50S, and 40 to 25S gradient regions, respectively. (C) Lanes 1 to 8 show the proteins coisolating in the 220 to 205S, 195 to 180S, 175 to 160S, 150 to 135S, 130 to 110S, 105 to 90S, 85 to 65S, and 60 to 45S gradient regions, respectively.

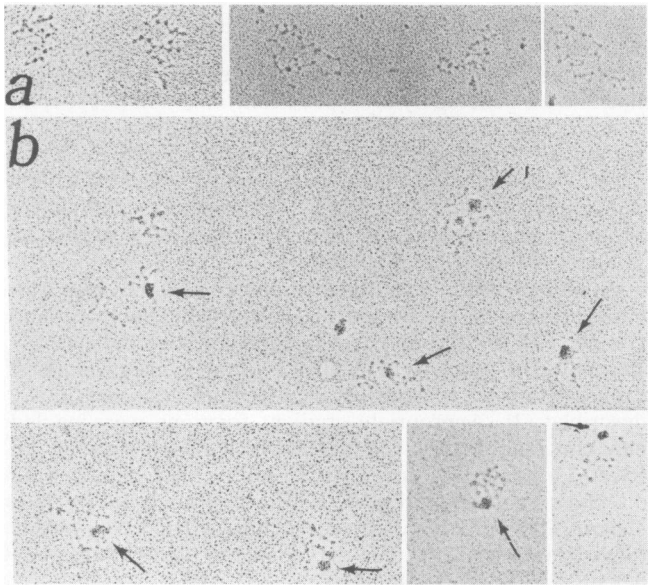

FIG. 4. The structure of *ts*BC216 nucleoproteins assembled in *ts*BC216-infected cells at 40°C. The 75S chromatin has the beads-on-a-string structure (a). The HSN obtained from the 100 to 120S gradient region is seen as chromatin to which a cluster of capsid proteins is attached (b).

Effect of temperature on the stability of *ts*BC nucleoprotein complexes. Studies conducted on the *ts*B group of mutants indicate that the partially assembled virions (100 to 160S) are the major species produced at the nonpermissive temperature (3, 3a, 5), whereas particles sedimenting at 220S are assembled when the infected cells are incubated under semirestrictive conditions (37°C) (16). These 220S complexes are not normal virions. V. Blasquez and M. Bina found that pulse-labeled 220S particles assembled at 37°C slowly dissociated as the incubation at 37°C was continued (unpublished observations). Stable 220S *ts*B virions were only produced at the permissive temperature (V. Blasquez and M. Bina, unpublished data).

We conducted similar experiments to gain insight into the effect of temperature on the stability of nucleoproteins assembled in cells infected with the *ts*BC group of mutants. Cells were infected with *ts*BC216 and labeled at 42.5, 40, or 37°C for 12 h before harvest. The nucleoproteins were extracted and analyzed. We found that at these temperatures only chromatin and HSN were assembled (Fig. 5). Thus, unlike the *ts*B group of mutants, 220S nucleoproteins were not produced in *ts*BC-infected cells at 37°C. This suggests that the mutations occurring in the BC region of VP1 may be more severe than those occurring in the B domain.

Blasquez et al. (5) further showed that the VP1 protein

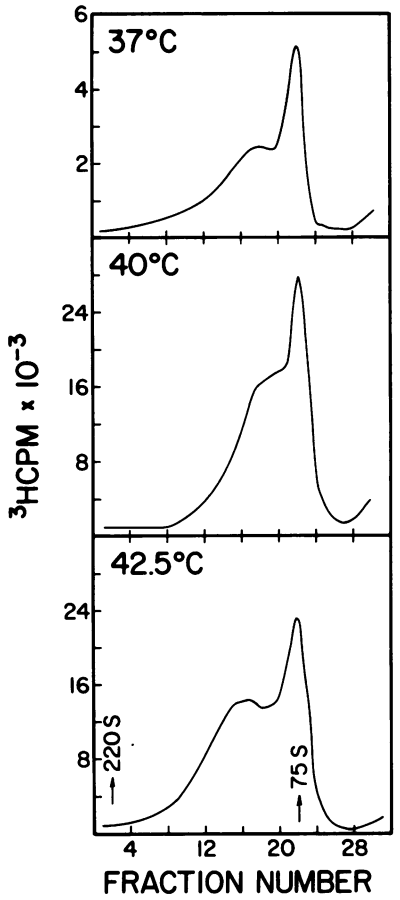

FIG. 5. Effect of temperature on nucleoproteins assembled in *ts*BC216-infected cells. The nucleoprotein complexes were isolated from *ts*BC216-infected cells labeled at 37, 40, or 42.5°C and analyzed by sedimentation in gradients of sucrose as described in the legend to Fig. 1.

produced at 40°C in cells infected with *ts*B mutants was used in virion assembly in vivo when these cells were shifted to the permissive temperature. Furthermore, the *ts*B virion assembly could be reversed by shifting the cells from the permissive temperature to the elevated temperature (Blasquez and Bina, unpublished observation). Because of our interest in the structure-function relationship of the VP1 protein, we performed similar temperature shift-up and shift-down experiments using cells infected with a mutant of the BC group.

First, we conducted experiments to follow the stability of the *ts*BC nucleoproteins assembled at 33°C after a shift-up to 40°C. Two sets of cells were infected with *ts*BC216 and incubated at 33°C. Both sets were pulse-labeled with [^{3}H]thymidine for 5 min and then were chased with excess cold thymidine for 8 h at the same temperature. Subsequently, one set was allowed to remain at 33°C as a control, and the other was shifted up to 40°C. Nucleoproteins were isolated from both sets 8 h later and were analyzed by sedimentation. Figure 6 shows that the 220S virions were produced and remained stable in cells incubated at 33°C. Virions were also isolated when the cells were harvested immediately after the 8-h chase. In contrast, we obtained primarily chromatin and HSN from cells which were labeled with [^{3}H]thymidine at 33°C and then shifted up to 40°C (Fig. 6A). These observations, taken together, indicate that the virions assembled at 33°C dissociate at 40°C, probably as a result of a change in the conformation of the temperature-sensitive protein.

We also conducted temperature shift-down experiments to follow the fate of the nucleoproteins assembled at 40°C. The experiments were performed as above, except that the cells were pulse-labeled and chased at 40°C. One set of infected cells was allowed to remain at 40°C as a control, and the other was shifted down to 33°C. The result of these experiments demonstrated that the pulse-labeled DNA remained as chromatin and HSN in cells incubated continuously at 40°C (Fig. 6B). In cells shifted down to 33°C, the pulse-labeled nucleoproteins matured to 220S virons (Fig. 6B). These results indicate that the *ts*BC216 nucleoproteins assembled at the nonpermissive temperature are used in virion production, via a pathway yet unknown, when they are incubated under permissive conditions in vivo. This could result from the renaturation of VP1 or a new round of VP1 synthesis at 33°C. To test the former possibility, we performed temperature shift-down experiments as above, except that we carried out the pulse-chase experiment with [^{3}H]leucine. The infected cells were pulse-labeled for 1 h with [^{3}H]leucine at 28 h postinfection at 40°C. This was followed by a 20-h chase

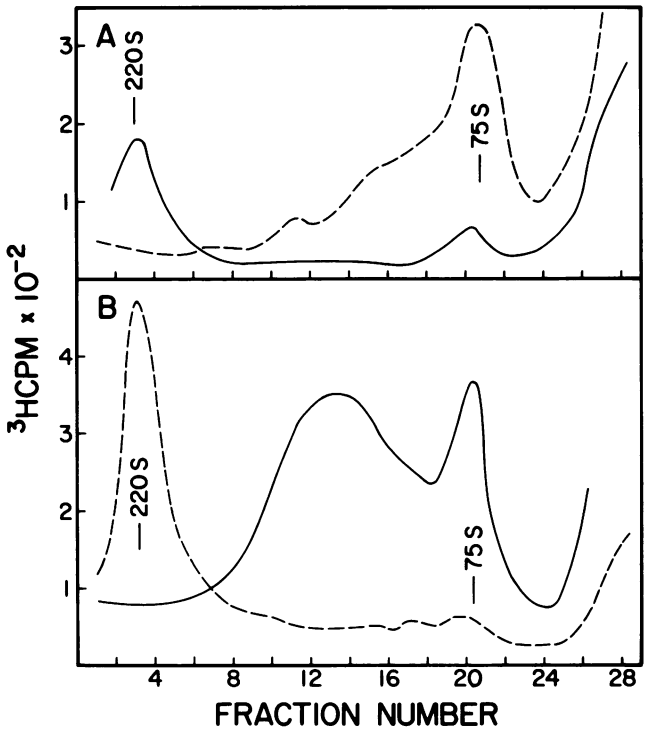

FIG. 6. Temperature shift-up and shift-down experiments. (A) Two sets of *ts*BC216-infected cells were pulse-labeled with [^{3}H]thymidine at the permissive temperature. After an 8-h chase with cold thymidine, one set was allowed to remain at 33°C (——), and other was shifted up to 40°C (– – – –). The nucleoprotein complexes were extracted from both sets 8 h later and were analyzed by sedimentation in sucrose gradients. (B) Two sets of *ts*BC216-infected cells were pulse-labeled with [^{3}H]thymidine at the restrictive temperature. After an 8-h chase with cold thymidine at the same temperature, the first was shifted down to 33°C (– – – –), and the other was allowed to remain at 40°C (——) as a control. After 8 h, the nucleoproteins were isolated from both sets and were analyzed as described above.

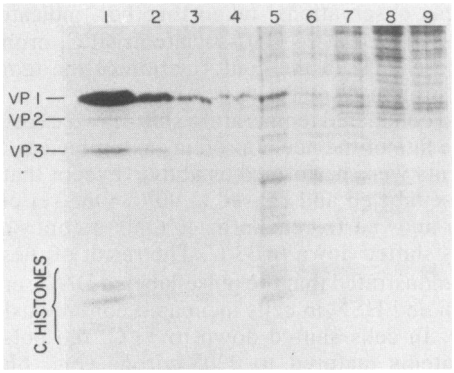

FIG. 7. Renaturation of VP1, synthesized at 40°C, after a shift-down to the permissive temperature. The *ts*BC216-infected cells were pulse-labeled with [^{3}H]leucine at 40°C and chased with unlabeled amino acids at the same temperature as described in the text. Subsequently, the cells were shifted down to 33°C for 20 h. Nucleoprotein complexes were isolated from these cells and sedimented in sucrose gradients. The appropriate gradient fractions were pooled, concentrated, and then analyzed for proteins by SDS-PAGE. Lanes show the labeled proteins in the various gradient regions of interest: 220 to 200S (lane 1), 190 to 170S (lane 2), 165 to 145S (lane 3), 135 to 120S (lane 4), 110 to 90S (lane 5), 85 to 65S (lane 6), 60 to 40S (lane 7), 35 to 15S (lane 8), 10 to 3S (lane 9).

with unlabeled amino acids at 40°C and then by incubation at the permissive temperature (33°C) for 24 h. Nucleoproteins were isolated from these cells and analyzed for proteins by SDS-PAGE. Figure 7 shows that the VP1 protein pulse-labeled at 40°C was a component of the virions assembled at 33°C. From this we conclude that the VP1 protein synthesized at 40°C renatures in vivo when the infected cells are incubated at the permissive temperature. In an independent series of experiments, we have found that this renaturation was less efficient as the incubation time at 40°C was increased during the chase period (data not shown).

DISCUSSION

Recent studies support the hypothesis that mature SV40 virions are produced via the gradual addition of the capsid proteins to viral chromatin (3a, 5, 9, 11–14; Bina et al., in press). An attractive feature of this model is the prediction that the capsid proteins should interact with SV40 chromatin in the course of virion assembly. Support for capsid-chromatin interactions during SV40 maturation comes from studies conducted by Coca-Prados and Hsu, who found that in electron micrographs, the wild-type previrion appeared as a shell associated with chromatin (9). Although we have obtained only a small amount of these structures from cells infected with a wild-type virus, we have observed that shell-chromatin complexes accumulate at 40°C in cells infected with the *ts*B group of SV40 assembly mutants (3, 3a, 5). Moreover, we have found that the shells attached to *ts*B chromatin are polymerized to various degrees (5), thus providing further support for the hypothesis that shell polymerization should proceed as the capsid proteins are added to chromatin (3a).

The possibility of direct interaction of capsid proteins with SV40 chromatin became even more evident when we analyzed the structure and the components of the nucleoproteins which accumulated at 40°C in cells infected with the *ts*BC group of SV40 assembly mutants. For example, Fig. 4 clearly shows that the *ts*BC216 nucleoproteins assembled at 40°C appeared primarily as chromatin and as chromatin to which a protein cluster was attached. These latter complexes sedimented more rapidly than 75S chromatin. SDS-PAGE analysis revealed that they contained the capsid proteins in addition to the cellular core histones (Fig. 2).

We have obtained similar types of nucleoproteins from cells infected with every mutant of the BC group except *ts*BC11 (Fig. 1). In agreement with the results obtained by Garber et al. (14), we observed that in *ts*BC11-infected cells, only 75S chromatin was produced (Fig. 1). In addition, we have tested the state of the VP1 protein produced in cells infected with selected mutants of the BC group (*ts*BC216, *ts*BC223, or *ts*B248) and found that a stable form of this protein is not only synthesized at 40°C but is also transported to the nuclei. Others have shown that the VP1 protein produced in *ts*BC11-infected cells is not stable at 40°C and is degraded in the cytoplasm (29). This instability may be related to the lack of assembly of intermediates other than the 75S chromatin in *ts*/BC11-infected cells under restrictive conditions (14). These results, taken together, indicate that *ts*BC11 is not a prototype member of the BC group of SV40 assembly mutants.

Our systematic characterization of a series of SV40 assembly mutants, shown here and elsewhere (3, 3a, 5) has led to a better understanding of the major capsid protein structure-function relationship. Thus far, our analysis is consistent with the hypothesis (20) that the VP1 protein may contain at least three domains. We have speculated that each of these domains may play a specific role in SV40 assembly (3a).

ACKNOWLEDGMENTS

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