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Histone Modifications in Simian Virus 40 and in Nucleoprotein Complexes Containing Supercoiled Viral DNA

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Simian virus (SV40) nucleoprotein complexes containing circular supercoiled viral DNA were extracted from infected cells and purified by differential centrifugation. The protein content of these complexes was compared by electrophoresis on 15% acrylamide gels with the protein content of purified SV40 virions and with histones from virus-infected cells. The electrophoretic patterns of histones from each of the sources revealed several major differences. SV40 virions contained histones H3, H2B, H2A, and H4 but not H1. Nucleoprotein complexes and host cells contained all five major histone groups. Relative to cellular histones, virion and nucleoprotein complex histones were enriched 15 to 40% in histones H3 and H4. In addition to the major classes of histones, several subfractions of histones H1, H3, and H4 were observed in acrylamide gels of proteins from SV40 virions and viral nucleoprotein complexes. Acetate labeling experiments indicated that each subfraction of histones H3 and H4 had a different level of acetylation. The histones from SV40 virions and nucleoprotein complexes were acetylated to significantly higher levels than those of infected host cells. No apparent differences in phosphorylation of the major histone groups were observed.

Nucleoprotein complexes (NPCs) containing viral DNA associated with proteins have been isolated from permissive cells infected with simian virus 40 (SV40) (13, 43) or polyoma virus (9, 11, 28, 31) and from the cores of mature virions (3-7, 15, 29). The proteins of NPCs and virion cores are primarily histones (1, 6, 16, 19, 32, 39, 40). Virion core proteins correspond to histones H3, H2A, H2B, and H4 (6, 14, 16, 20, 24, 25) whereas NPCs contain each of the latter groups plus histones H1 (18, 37, 39, 40).

Although histones exhibit extreme evolutionary stability in their primary structure, they acquire considerable heterogeneity through post-translational modifications such as acetylation and phosphorylation (23, 34). These modifications are correlated with changes in transcriptional, structural, and replicative properties of the cellular genome. The possibility that similar mechanisms may operate in papovavirus "mini-chromosomes" is suggested by recent evidence that polyoma and SV40 virions contain modified histones (26, 30, 35). In two of these reports (30, 35), a high degree of acetylation of virion histones H3 and H4 was inferred from their electrophoretic complexity. However, these conclusions were not confirmed by more definitive techniques such as acetate labeling of histone subfractions. Therefore, the purpose of this study was to determine whether the electrophoretic complexity of SV40 virion histones was correlated with specific degrees of acetylation. An additional aim was to determine whether SV40 nucleoprotein complexes from virus-infected cells contained similarly modified histones.

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MATERIALS AND METHODS

Virus and cells. SV40 (strain RH911) was propagated in the TC-7 clone of the CV-1 monkey kidney cell line. Cell monolayers were grown to confluence on 10-cm plastic petri dishes containing 10 ml of Eagle medium supplemented with 10% calf serum. Cultures were infected approximately 24 h after confluence with 0.5 ml of virus (1 to 2 PFU/cell), and the virus was allowed to adsorb for 90 min. After adsorption, 10 ml of Eagle medium containing 2% horse serum was added to each culture.

Isotopic labeling and virus purification. Immediately after transfer, 10 ml of Eagle medium containing 10% calf serum and either 5.0 μCi of ^{3}H-amino acid mixture per ml or 2.5 μCi of [^{14}C]acetate per ml

(58 mCi/mmol) was added to the cells, and the cultures were incubated until they had been confluent for 24 h. At 24 h postconfluence, labeling medium was removed and cells were infected as described above. After infection, cells were overlaid with Eagle medium containing 2% horse serum and either 5 μCi of ^{3}H-amino acid mixture per ml or 2.5 μCi of [^{14}C]acetate acid per ml. For ^{32}P-labeling experiments, carrier-free [^{32}P]-phosphate was added to each culture (10 μCi/ml) 24 h after infection as described by Tan and Sokol (37).

Approximately 7 days postinfection, when infected monolayers were 80 to 90% lysed, cultures were frozen and thawed three times. Lysates were pooled and clarified by centrifugation for 20 min at 10,000 rpm in a Sorvall RC-2B centrifuge (SS-34 rotor). The supernatant fluids and pellets were retained for additional purification. Pellets were suspended in 2.5 ml of Eagle medium, an equal volume of 1,1,2-trichlorotrifluoroethane was added, and the solutions were homogenized on a Vortex mixer at maximum speed for 4 to 5 min. Phases were separated by centrifugation at 1,000 rpm in an IEC HN-S centrifuge, and the top aqueous phase, containing SV40 virions, was combined with the previously collected supernatants (8). Virus was pelleted from the pooled supernatant fluids by centrifugation at 25,000 rpm for 3 h at 5°C in a Spinco type 30 rotor. Pellets were suspended in CsCl (density 1.33 g/ml in 0.02 M EDTA) and centrifuged to equilibrium at 35,000 rpm for 48 h at 25°C in a Spinco SW50.1 rotor. The peak fractions of the band containing complete virions (lower band) were pooled and dialyzed against 0.2 M Tris (pH 7.0). The dialyzed virus was layered onto either a 15.0-ml CsCl-sucrose step gradient (5.0 ml of CsCl, density 1.38 g/ml in 0.2 M Tris-hydrochloride [pH 7.0], and 10.0 ml of 20% [wt/vol] sucrose in 0.2 M Tris [pH 7.0] as described by Schaffhausen and Benjamin [30]) or a CsCl-sucrose step gradient consisting of 1.0 ml of the CsCl solution and 3.5 ml of the sucrose solution. The former gradient was centrifuged at 25,000 rpm for 3 h at 20°C in a Spinco SW27 rotor, and the latter gradient was centrifuged at 30,000 rpm for 3 h at 20°C in a Beckman SW50.1 rotor. The lower band of virions was collected, dialyzed against 0.001 M Tris (pH 7.9), lyophilized, and stored at 4°C in a vacuum desiccator.

Isolation of viral nucleoprotein complexes. NPCs containing SV40 DNA were extracted by procedures described previously (9, 13). Approximately 42 h after infection, monolayers were washed twice with ice-cold Tris-buffered saline, and each culture was treated with 0.9 ml of a solution containing 0.25% Triton X-100, 0.01 M EDTA, and 0.01 M Tris (pH 7.9; TTE) (11). After 10 min at room temperature, 0.1 ml of 1.5 M NaCl was added to each petri dish and they were maintained at 4°C for an additional 3 h. This treatment lysed cells, leaving nuclei morphologically intact. Lysates were poured into centrifuge tubes, and nuclei and large cellular debris were pelleted by centrifugation at 2,000 rpm for 5 min at 4°C in an IEC HN-S centrifuge (no. 958 rotor). The supernatant fluid (Triton cytoplasmic fraction) containing SV40 NPC was decanted and stored at 4°C. This crude supernatant fraction was concentrated to approximately one-fifth its original volume by vacuum dialysis against TTE using a Sartorious collodion bag apparatus. Initial purification of the concentrated extract was accomplished by velocity centrifugation of 0.5-ml samples in 10.01-ml, linear, 10 to 30% (wt/wt) sucrose gradients prepared in STTE buffer (TTE buffer containing 0.2 M NaCl). Samples were centrifuged at 35,000 rpm for 165 min at 4°C in a Spinco SW41 rotor. Tubes were punctured at the bottom, and 35 to 40 fractions were collected. Aliquots (25λ) of each fraction were transferred to filter disks, and trichloroacetic acid-precipitable radioactivity was determined. Fractions corresponding to the 61S supercoiled form NPC (NPC-SC) (13) were pooled and diluted to approximately 10% sucrose by addition of STTE. The diluted NPC-SC preparation was further purified by layering 2.0-ml samples onto sucrose gradients consisting of 2.5 ml of 20% (wt/wt) sucrose in STTE over a cushion of 0.5 ml of 60% (wt/wt) sucrose in STTE. These gradients were centrifuged at 40,000 rpm for 16 h at 4°C in a Spinco SW50.1 rotor. The pellet containing SV40 NPC was either dissolved in STTE or the electrophoresis buffer described in a succeeding paragraph.

The efficacy of this purification procedure is based upon separation of replicative form NPC (NPC-RF) and NPC-SC by differences in their sedimentation velocities in the first gradient, followed by removal of contaminating ribosomes, primarily from NPC-SC fractions, by the second gradient. The ribosomal contaminants remain in the 20% sucrose because, during the 3- to 4-day purification sequence, ribosomes are dissociated due to the presence of EDTA in all buffers (13, 38; D. A. Goldstein, and M. R. Hall, unpublished data).

Histone extraction. Confluent TC-7 monolayers were lysed by the procedure previously described for NPC extraction (9, 13), and nuclei were separated from soluble cytoplasmic components by centrifugation at 2,000 rpm for 5 min in the no. 958 swinging-bucket rotor of an IEC HN-S centrifuge. The pellet, containing TC-7 nuclei, was retained and histones were extracted utilizing 0.4 N H_2SO_4 as described by Panyim et al. (21). Histone concentrations in selected samples were determined by the method of Lowry et al. (17) using calf thymus histones as standards.

Polyacrylamide gel electrophoresis. Proteins were resolved by electrophoresis in 15% acrylamide gels containing 0.9 N acetic acid and 2.5 M urea as described by Panyim and Chalkley (22). Histones, purified virions, or NPCs were dissolved in a dissociation buffer consisting of 10 M urea, 2% 2-mercaptoethanol, 5 mg of protamine per ml, 15% sucrose (wt/vol), and 0.9 N acetic acid. After incubation at 37°C for 16 h, 50- to 100-μl samples were mixed with methyl green tracking dye and layered on the gels.

Relative proportions of proteins were determined by either dye content or radioactivity of the protein bands. Gels were scanned at 630 nm by a Gilford spectrophotometer gel scanner with an attached linear transport. Measurement of peak sizes was then used to determine relative stain content of each band. Alternatively, each band was excised from the gel, and the dye was removed with 2.0 ml of dimethylsulfoxide at 37°C overnight (27). Optical density readings of the extracts were made at 615 nm in a Beckman model 25 spectrophotometer. Since amido black binds to each class of histones with a different affinity, the dye

content values were adjusted by binding factors determined by Sonnenbickler and Zetl (33).

Radioactivity of protein bands was determined by either excising the whole band or by slicing the frozen gel into 1-mm disks with a Biorad model 190 gel slicer. The excised bands or the individual gel slices were solubilized in 0.5 ml of 30% H_2O_2 (vol/vol) for a minimum of 24 h at 37°C, and then 10 ml of Yorktown TT21 scintillation fluid was added for scintillation counting.

Centrifugal analyses of NPC and viral DNA. Sedimentation characteristics of various samples of viral DNA and NPCs were determined on linear 5 to 20% (wt/wt) sucrose gradients as previously described (9, 13).

Supercoiled and replicating SV40 DNA molecules were separated from open circular and linear forms by equilibrium centrifugation in CsCl solutions containing propidium diiodide as described in previous reports (9, 13).

The buoyant density of samples of purified NPC was determined by centrifugation of glutaraldehyde-fixed complex in preformed linear CsCl gradients as previously reported (9, 13).

Chemicals. Chemicals were obtained from the following sources: ^{3}H-amino acid mixture, ^{14}C-amino acid mixture, [^{32}P]phosphate, [*methyl*-^{3}H]thymidine (55 to 60 Ci/mmol), and [*methyl*-^{14}C]thymidine (54 to 57 mCi/mmol) were from Schwarz/Mann, Orangeburg, N.Y.; [^{14}C]acetic acid as sodium salt (58 mCi/mmol) was from Amersham/Searle, Arlington Heights, Ill.; Triton X-100 was from Sigma Chemical Co., St. Louis, Mo.; propidium diiodide was from Calbiochem (San Diego, Calif.); glutaraldehyde was from Aldrich Chemical Co., Milwaukee, Wis.; CsCl and sucrose were from Schwarz/Mann, Orangeburg, N.Y.; Eagle minimal essential medium was from K. C. Biological, Inc., Lenexa, Kan.; and TT-21 scintillation fluid was from Yorktown Research, Hackensack, N.J.

RESULTS

Purification of nucleoprotein complexes. For these experiments, cells were prelabeled with ^{3}H-amino acid mixture for 96 h prior to infection. Immediately after infection, cultures were again overlaid with medium containing ^{3}H-amino acid mixture until NPCs were extracted. In addition, from 38 to 42 h postinfection, cultures were labeled with [^{14}C]thymidine. Cells were lysed and NPCs were extracted from these cultures approximately 42 h after infection. Figure 1 represents the sedimentation profile of these extracts in preparative 10 to 30% sucrose gradients. The peak of [^{14}C]thymidine-labeled material present in fraction 23 has the sedimentation coefficient (61S) expected of SV40 NPCs (13). Most of the ^{3}H-amino acid radioactivity found at the top of the gradient presumably consists of cellular proteins. However, the peak of ^{3}H-labeled material at 45 to 47S (fractions 30 to 33 has previously been shown to consist of ribonucleoprotein [13]).

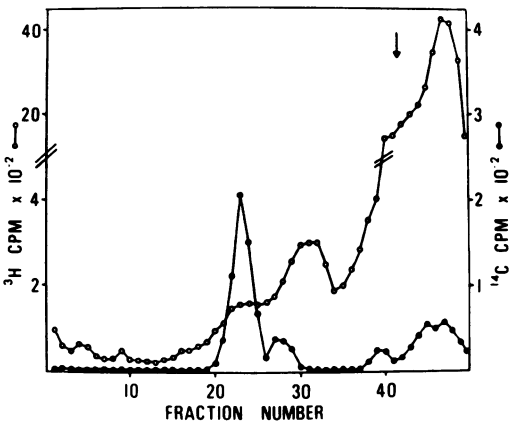

FIG. 1. *Velocity sedimentation of SV40 NPCs in 10 to 30% sucrose gradients. Monolayers were labeled with ^{3}H-amino acids mixture (5 μCi/ml) for 96 h prior to infection and for 42 h after infection. At 38 h postinfection, [^{14}C]thymidine was added to the cultures to a final concentration of 1 μCi/ml. NPCs were extracted and concentrated as described in the text. Samples (0.5 ml) of the concentrated extract were layered on 10.0-ml sucrose gradients and centrifuged as described in the text. The arrow indicates the position of a ^{14}C-labeled SV40 DNA marker sedimented in separate tubes. Sedimentation is from right to left.*

To insure that the 61S nucleoprotein complex containing NPC-SC was separated from the 70S nucleoprotein complex containing NPC-RF and from the trailing fractions of ribonucleoprotein, fractions 20 to 26 were pooled and centrifuged in 20 to 60% sucrose step gradients (Materials and Methods). A loose pellet of NPC was recovered from these gradients and utilized for further analysis.

Analysis of ^{3}H-amino acid-labeled material, isolated as described above on 5 to 20% sucrose gradients, reveals that essentially all of the non-NPC-associated radioactivity has been removed (Fig. 2).

To determine whether the DNA in purified NPC preparations was in a supercoiled form, an aliquot of purified NPC was treated with 0.1% sodium dodecyl sulfate to remove associated proteins. The deproteinized samples were then mixed with a [^{14}C]thymidine-labeled viral DNA marker and analyzed by equilibrium centrifugation in CsCl-propidium diiodide gradients. Over 95% of the total tritium counts found in these gradients were associated with the band of NPC-SC.

The average density reported for glutaraldehyde-fixed SV40 NPCs is 1.437 ± 0.008 g/cm^3 (13). Density analyses of glutaraldehyde-fixed purified samples of NPC-SC were conducted on preformed linear CsCl gradients as previously

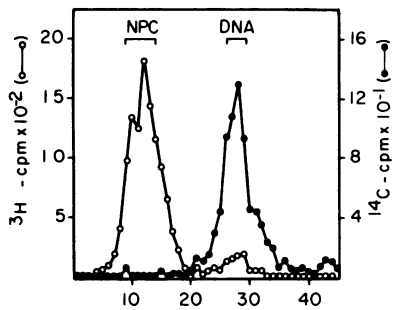

FIG. 2. *Velocity sedimentation of purified SV40 NPCs in 5 to 20% sucrose gradients. Monolayers were labeled with ^{3}H-amino acids mixture (5 μCi/ml) for 96 h prior to infection and for 42 h after infection. NPCs were extracted and concentrated as described in the text. The crude concentrated extract was partially purified on 10 to 30% sucrose gradients as described in Fig. 1. Fractions in the NPC peak were pooled and centrifuged in 20 to 60% sucrose gradients as described in the text. Aliquots (100λ) of NPC-SC from these gradients were mixed with [^{14}C]thymidine-labeled viral DNA marker and centrifuged as described in the text. Sedimentation is from right to left.*

described (9, 13). In all samples studied the purified NPC preparations exhibited buoyant densities within the range expected for SV40 NPCs.

Analysis of SV40 virion and NPCs. Cellular histones, purified virions, and purified NPC-SC were treated with dissociating buffer, and their proteins were analyzed by gel electrophoresis. The upper section of gels containing SV40 virions and NPC-SC contained coat proteins and several additional unidentified proteins. Nonstructural nonhistone proteins are also apparent in gels of NPC-SC. The botton portions of all gels contained proteins which coelectrophoresed in correspondence with purified calf thymus histone fractions used as markers.

Since there appeared to be several subfractions within each histone group, close-up photographs were taken of the histone region of each gel (Fig. 3). In the gel containing extracted TC-7 histones, all five major classes of histones were well separated. From top to bottom of the gel they are H1 (three subfractions), H3, H2B, H2A, and H4 (two subfractions). The gel containing SV40 virion proteins contained bands corresponding to histone groups H3, H2B, H2A, and H4; however, histone H1 was not detected. At least four subfractions are apparent in the H3 and H4 histone regions of the SV40 gel. It has been demonstrated with HeLa cell histones that these subfractions represent different degrees of acetylation (41). All five major histone groups were found in the gel of NPC-SC. From top to bottom of the gel they are H1 (four subfractions), H3 (three subfractions), H2B, H2A, and H4 (three subfractions). In some preparations of NPC-SC the H3 histone group appeared to be represented by five subfractions rather than the four subfractions shown in Fig. 3, whereas H4 could contain four subfractions rather than the three shown in Fig. 3.

Higher-resolution analyses of histone subfractions were also made from densitometer scans of the histone regions of amido black-stained gels (Fig. 4). Although the various histone peaks are more apparent in scans than in the photographs, in general the gel scans support the data described above from visual analyses of gel photographs.

In addition to the identifable histone fractions, there are three unidentified bands which have been designated x, y, and z, respectively, in Fig. 3. Proteins y and z were present consistently in gels of SV40 virion proteins. Proteins migrating in similar positions have previously been demonstrated in polyoma virus (30). Bands x and z were consistently found in gels of NPC-SC.

Quantitation of histone fractions. Quantitation of the various histone fractions in acrylamide gels was accomplished by scanning stained gels and determination of the weights of the scanned histone peaks, and by determination of the total radioactivity in each peak of labeled histones (Table 1). Histone H2B was generally

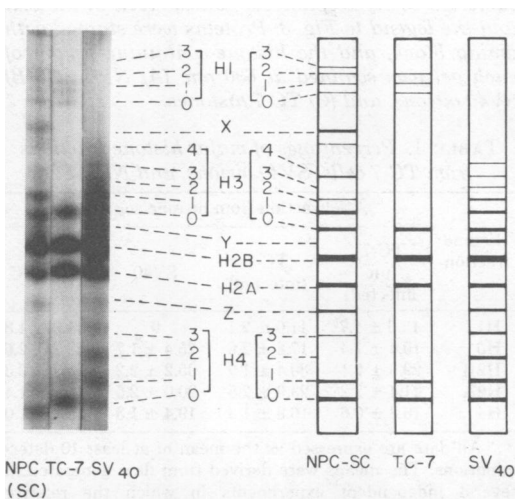

FIG. 3. *Polyacrylamide gel electrophoretic analysis of the histones from SV40, NPC-SC, TC-7 cells, and SV40 virions. Samples were prepared for electrophoresis as described in the text. Polypeptides were separated on 22-cm 15% acrylamide gels as described in the text. Gels were stained with amido black and photographed on Kodak Panatomic X film using a red filter. Only the gel segments containing histones were photographed for this figure. Diagrammatic representations of each gel are presented to the right.*

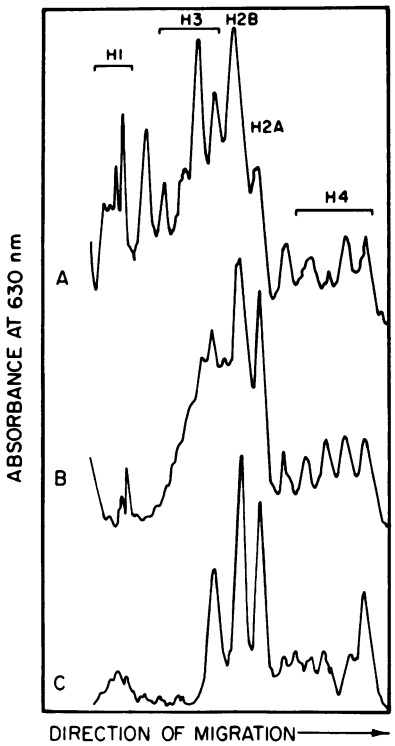

FIG. 4. *Densitometric scans of the histone regions of polyacrylamide gels of SV40 NPC-SC, SV40 virions, and TC-7 cells. Samples were prepared and subjected to electrophoresis as described in the text and the legend to Fig. 3. Proteins were stained with amido black, and the histone-containing region of each gel was scanned at 630 nm. (A) NPC-SC, (B) SV40 virions, and (C) TC-7 histones.*

TABLE 1. *Percentages of major histone fractions from TC-7 cells, SV40 virions, and NPC-SC*

Histone fraction	% Fraction from histone source			
	TC-7[a] (uninfected)	TC-7 (infected)	SV40	NPC-SC
H1	13.6 ± 1.7[b]	11.0 ± 2.1	0	11.9 ± 1.8
H3	19.4 ± 1.4	17.8 ± 2.6	25.4 ± 1.7	27.2 ± 2.6
H2B	29.3 ± 2.1	30.4 ± 1.9	35.2 ± 2.2	31.2 ± 2.3
H2A	21.4 ± 2.2	23.9 ± 2.5	20.0 ± 2.5	16.5 ± 1.4
H4	16.3 ± 0.6	16.9 ± 1.3	19.4 ± 1.3	13.2 ± 1.0

[a] All data are expressed as the mean of at least 10 determinations. The means were derived from data generated in several independent experiments in which the relative amounts of each histone group were determined by integration of peaks in spectrophotometric scans of acrylamide gels, by spectrophotometric determination of bound dye, and by scintillation counting of excised histone bands from acrylamide gels.

[b] Mean ± standard error.

the predominant species isolated from TC-7 cells. Relative to cellular histones, virions and NPC-SC samples were enriched in histone H3. In addition, the ratios of arginine-rich to lysine-rich histones, i.e., H3 + H4/H2B + H2A, are higher in virions and nucleoprotein complexes. This indicates that virions and NPCs are enriched in the two histone groups (H3 and H4) believed to comprise the internal cores of nucleosomes (42).

Quantitation of histone subfraction acetylation. The patterns of histone subfractions observed in Fig. 3 and 4 are similar to those previously interpreted as representative of different degrees of acetylation (30, 35, 41). Visually, it also appears that there are quantitative differences in these subfractions between histones isolated from TC-7 cells, SV40 virions, and NPC-SC. By labeling with [^{14}C]acetate it was possible to compare the total counts in each histone subfraction with that subfraction's relative concentration and thus derive a relative specific acetylation activity for each histone band (Table 2). The subfractions of each histone class were numbered from the band with the highest electrophoretic mobility to the lowest. In NPC-SC and SV40 virions, the subfractions of histones H3 and H4 exhibit increasing specific activities of [^{14}C]acetate in order of decreasing electrophoretic mobility. For example, examination of specific activities of the H4 subfractions of the SV40 virion histones reveals that the fastest-migrating band has the most protein and the lowest specific activity. The second-fastest-migrating H4 subfraction has a higher specific activity and migrates in the expected position for a monoacetylated form of H4. The third

TABLE 2. *Specific activities of [^{14}C]acetate-labeled histone subfractions from NPC-SC, TC-7 cells, and SV40 virions*

Subfraction	Sp act[a]		
	NPC-SC	TC-7	SV40 virion
H1			
3	4.82		
2	4.50	2.58	
1	2.73	1.90	
0	4.83	2.51	
H3			
3	2.07		1.79
2	1.91		1.49
1	0.77		1.17
0	0.71	0.63	0.87
H2B	0.67	0.94	0.93
H2A	0.66	0.69	1.09
H4			
3	2.46	1.19	1.08
2	2.25	1.07	1.00
1	0.87	0.84	0.99
0	0.76	0.76	0.91

[a] Specific activity = (subfraction counts per minute [cpm] as percentage of total cpm) divided by (subfraction weight as percentage of total weight).

and fourth bands have even higher specific activities, indicating a higher degree of acetylation, and migrate in the expected positions for diacetylated and triacetylated forms of H4, respectively. A similar pattern of increasing specific activities with bands of decreasing mobility is evident in the subfractions of histone H3. Corresponding histone subfractions from NPC-SC exhibit similar patterns of specific activities with labeled acetate (Table 2).

Although histone H1 was electrophoretically heterogeneous (Table 2), the degrees of acetylation of subfractions appear to be random.

Quantitation of histone subfraction phosphorylation. The degree of phosphorylation of each histone fraction was examined by adding carrier-free [^{32}P]phosphate, in a series of experiments similar to those described in the preceding section (Table 3). No specific patterns of phosphorylation could be found among the subfractions of histone H1, H3, or H4. However, it should be noted (Table 3) that in all cases the ^{32}P-specific activities of H1 subfractions are higher than those of the other histones.

DISCUSSION

Electrophoretic analyses of the core histones of polyoma and SV40 virions reveal extensive histone complexity (30, 35). It has been proposed (30, 35, 41) that the complex electrophoretic patterns of histones H3 and H4 are attributable to varying degrees of acetylation. Results reported here confirm the report of electrophoretic complexity of SV40 virion histones (35). In addition, the distribution of radioactivity in [^{14}C]acetate-labeled virion histone subfractions lends strong support to the proposal that the complex electrophoretic banding patterns of H3 and H4 are a result of extensive acetylation. In agreement with the results of Tan (35), histones incorporated into virions were enriched in H3 and H4.

TABLE 3. *Specific activities of [^{32}P]phosphate-labeled histone subfractions from NPC-SC, TC-7 cells, and SV40 virions*

Subfraction	Sp act[a]		
	NPC-SC	TC-7	SV40 virion
H1			
3	2.64		
2	2.84	3.57	
1	4.02	3.61	
0	1.96	2.51	
H3			
3	1.34		1.61
2	0.52		1.02
1	0.61		1.47
0	0.74	0.89	1.20
H2B	0.28	0.56	0.87
H2A	0.39	0.42	0.68
H4			
3	0.86	0.90	1.07
2	0.82	1.05	0.75
1	1.45	0.84	1.63
0	0.90	0.64	1.56

[a] See Table 2, footnote *a*.

Of particular importance are the observations that viral nucleoprotein complexes extracted from infected cells contain all five major histone groups, with H1, H3, and H4 exhibiting marked heterogeneity. Although several authors have reported the presence of the five major histone groups in viral NPCs (1, 6, 14, 25, 39, 40), data presented here represent the initial report of apparently modified histones in SV40 NPC-SC extracted from infected cells. Acetate labeling experiments indicate that the observed electrophoretic subfractions of histones H3 and H4 contain differing levels of acetylation.

It has been suggested that histones possess the potential for modulating gene activity (23, 34). Currently, histones H3, H2A, H2B, and H4 are considered to interact with DNA in the form of tetrameric complexes called nucleosomes (42). The modulatory function of histone probably occurs via changes in nucleosome structure induced by histone modifications such as acetylation and phosphorylation. Specifically, acetylation of histones H3 and H4 are postulated to facilitate nucleosome modifications required for DNA replication and transcription (42). The extensive modification of histones H3 and H4, previously reported for polyoma and SV40 virion histones (26, 30, 35) and described here for SV40 virions and NPC-SC, strongly suggests that these viral minichromosomes are in a biosynthetically active state. This prediction is supported by the findings of Birkenmeier et al. (2) that a viral NPC containing covalently closed circular viral DNA (DNA I) serves as the template for transcription during the late phase of lytic infection. In addition, we have recently reported that the SV40 NPCs containing DNA I are transcriptionally active when incubated with *Escherichia coli* DNA-dependent RNA polymerase (12), whereas others have described procedures for transcription of NPCs with endogenous RNA polymerase II (4, 10).

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