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Acetylation of Histone-Like Proteins of Adenovirus Type 5

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We examined acetylation of the histone-like adenovirus core proteins VII and V and the precursor of the major core protein, pVII, by measuring the incorporation of [^{14}C]acetate. Adenovirus proteins pVII and V appeared to be acetylated, whereas protein VII was not. Label incorporated into these viral proteins in the form of acetate was metabolically stable, and labeling was not enhanced by treatment with sodium butyrate, an inhibitor of histone deacetylases. Viral protein acetylation therefore differs from the reversible acetylation of histones that has been implicated in transient alterations of chromatin structure. Inhibition of protein synthesis in infected cells resulted in a proportional reduction in [^{14}C]acetate uptake into pVII and V, suggesting that these proteins undergo acetylation during protein synthesis and not as a post-translational modification. Therefore, these viral proteins are probably acetylated amino-terminally, a characteristic shared by three of the five major histone classes.

Adenoviruses replicate and transcribe their DNA in host nuclei by using the synthetic machinery of the host cells (15, 44), although several lines of evidence suggest that adenoviral chromatin differs from normal eucaryotic chromatin, at least during some phases of the virus replication cycle. For these reasons, adenovirus chromatin provides an intriguing contrast to cellular chromatin as an alternative type of DNA-protein complex which functions in eucaryotic cells.

The DNA of eucaryotic cells is arranged with small, basic histones in repeating units called nucleosomes. The nucleosome core, an approximately spherical particle having a diameter of 10 nm, consists of a globular cluster of two each of histones H2A, H2B, H3, and H4 and 140 base pairs of DNA, resulting in about five-fold compaction of the DNA. The linker region connecting adjacent nucleosome cores contains about 60 base pairs of DNA and may be associated with histone H1 (14). Sequence analyses of the histones have revealed basic domains, which are believed to be involved in interactions with DNA, and hydrophobic and acidic domains, which may participate in interactions among histones and between histones and other chromosomal proteins (19).

Unlike simian virus 40 and polyoma virus, which package viral DNA with cellular histones, adenovirus encodes its own histone-like proteins, which are present in association with the DNA within the virus particles. The minor core protein, protein V (molecular weight, 45,000), resembles histones in containing 18% lysine and arginine residues. The major core protein, protein VII, has a molecular weight of 18,000 and contains 25% lysine and arginine residues (25, 30, 31, 37, 40). The basic residues of core protein VII are distributed in a way similar to the distribution in histones H3 and H4, that is, predominantly within the amino-terminal portion of the protein, which may constitute a DNA-binding domain similar to those of histones (28, 42). The combined mass of core proteins V and VII within the virion is equal to the mass of the viral DNA.

It is this DNA-protein complex that penetrates the nucleus of a host cell upon infection. Sedimentation analyses of the fate of adenoviral DNA during virus uncoating have revealed no difference in sedimentation coefficients between naked DNA and the endproduct of uncoating (5, 29). However, examinations of the protection of parental viral DNA from nuclease digestion at early times in infection have shown an extent of protection greater than that expected for naked DNA. The kinetics of digestion are similar to those observed in eucaryotic chromatin, and protected fragments of DNA are similar in size to the fragments observed in digested viral cores (9, 41). The role of adenovirus core proteins in the substrate for early virus transcription remains unclear.

The major core protein, protein VII, is synthesized in the form of a 20,000-molecular-weight precursor, pVII (6). Twenty additional amino acid residues which are present at the amino terminus of pVII have been found to include hydrophobic and possibly acidic residues. The sequence of the precursor portion of pVII has not been determined due to the presence of a blocked amino acid at the amino terminus, which is not available for dansylation or reaction with phenylisothiocyanate in sequenc-

ing reactions (28).

Synthesis of pVII begins concurrently with the onset of viral DNA synthesis, which defines the late phase of infection, during which cellular protein synthesis, including histone synthesis, is greatly reduced (6, 40, 43). Electron microscopy of infected cell nuclei late in infection has revealed smooth fibers with the dimensions of adenovirus DNA and no evidence of a nucleosomal repeat (32). Whether adenovirus protein pVII contributes to the structure of replication and transcription complexes late in infection remains unclear. pVII has been found to be associated with intermediates in virus assembly, and cleavage of pVII into VII occurs as a step in the condensation and packaging of the viral DNA into the virus capsid (4, 11–13, 20).

Certain chemical modifications of cellular histones, specifically acetylation, correlate in a general fashion with changes in chromatin activity (2, 3, 8, 33, 39). The reversible acetylation of internal lysine residues, which alters the charge of the histones, is believed to modulate the affinity of histones for DNA, thereby facilitating transcription, chromatin assembly, and chromatin condensation. In the case of adenoviruses, the timing of the synthesis of various chromatin components can be correlated with specific switches in transcription and DNA replication; pVII and V syntheses are concurrent with viral DNA synthesis, and pVII-to-VII processing accompanies viral DNA condensation and packaging. It was the intent of this investigation to determine whether protein modification through acetylation is also a feature of the adenovirus chromatin system.

MATERIALS AND METHODS

Cells, virus, and viral infections. Adenovirus type 5 was grown in a HeLa S3 suspension culture in Spinner minimal essential medium (medium F13; GIBCO Laboratories) supplemented with 5% calf serum, penicillin, and streptomycin. Infected cells were washed with phosphate-buffered saline, disrupted by three cycles of freezing and thawing, sonicated, and extracted with freon. The resulting lysate was titrated and used for infections. Infections were carried out with confluent HeLa monolayers on 100-mm petri dishes at a multiplicity of 50 PFU/cell. Virus was allowed to adsorb to cells for 1 h at 37°C. After infection, cultures were maintained in the Dulbecco modification of Eagle medium (DME) (Flow Laboratories) supplemented with 5% fetal calf serum, penicillin (50 IU/ml), and streptomycin (50 μg/ml).

Radioactive labeling and drugs. Cells were labeled with 1 μCi of L-[*U*-^{14}C]arginine (298 mCi/mmol) per ml, 10 μCi of [^{14}C]sodium acetate (58.7 mCi/mmol) per ml, 16 μCi of L-[2(n)-^{3}H]methionine (4.9 Ci/mmol per ml, or 0.6 μCi of L-[5(n)-^{3}H]arginine (8 to 25 Ci/mmol) per ml for the times specified in the figure legends. In experiments with both [^{14}C]sodium acetate and [^{14}C]arginine, DME containing 5% dialyzed fetal calf serum was used as the labeling medium. DME minus methionine but containing 5% dialyzed fetal calf serum was used for experiments in which both [^{3}H]-methionine and [^{14}C]sodium acetate were used.

Cold chases were performed by washing cell monolayers three times with prewarmed DME containing 5% fetal calf serum, followed by incubation in DME containing 5% fetal calf serum; 7.5 mM sodium butyrate was added where noted.

Where indicated, cycloheximide was added to the culture medium at a concentration of 30 μg/ml for a period extending from 30 min before the addition of label to the end of the labeling period.

Acid-soluble protein extraction and gel electrophoresis. The extraction of nuclei with sulfuric acid, followed by acetone precipitation, has been described previously (24). The acid-extractable proteins from one 100-mm plate were suspended in 50 μl of acid-urea sample buffer (15% [wt/vol] sucrose, 2.5 M urea, 0.9 N acetic acid, pH 2.7) before electrophoresis on the acid-urea gel system of Panyim and Chalkley (34), which was performed as described previously (24).

Triton X-100–acid–urea gel electrophoresis (1; A. Zweidler and L. H. Cohen, Fed. Proc. **13**:926, 1972) was performed on acid-extractable proteins suspended in Triton X-100–acid–urea sample buffer (15% [wt/vol] sucrose, 0.6 M urea, 0.37% [vol/vol] Trition X-100, 0.9 N acetic acid [pH 2.7], 5% [vol/vol] β-mercaptoethanol). Slab gels (0.15 by 10 by 14 cm) were prepared in the following manner. A 0.03-g amount of ammonium persulfate was dissolved in 15 ml of 9.6 M urea–0.59% (vol/vol) Trition X-100 and added to a solution containing 6.0 ml of 60% (wt/vol) acrylamide–0.4% (wt/vol) *N,N'*-bisacrylamide in water and 3.0 ml of 43.2% (vol/vol) glacial acetic acid–4.0% *N,N,N',N'*-tetramethylenediamine in water, resulting in a final composition of 15% acrylamide, 0.1% bisacrylamide, 6 M urea, 0.37% Triton X-100, and 0.9 N acetic acid (pH 2.7). Gels were pre-electrophoresed at 20 mA until the voltage remained constant. Before sample application, 50 μl of 0.1 M cysteamine–15% (wt/vol) sucrose–0.6 M urea–0.37% (vol/vol) Triton X-100–0.9 N acetic acid was added to each sample well; 140 V was applied for 30 min, and residual cysteamine was removed. Samples (10 to 20 μl) were electrophoresed for 13 h at a constant current of 13 mA and 110 V. After electrophoresis, gels were stained with 0.5% (wt/vol) amido black 10B–7.5% (vol/vol) acetic acid for 30 min and destained in 35% (vol/vol) ethanol–7% (vol/vol) acetic acid.

Viral proteins were identified on the Triton X-100–acid–urea gel system by electrophoresis of purified pVII and VII, which were kindly supplied by Michael Sung. Histones were identified on the Triton X-100–acid–urea gel system by electrophoresis of histone bands cut from an acid-urea gel. After electrophoresis of proteins which were acid extracted from infected nuclei by using the acid-urea gel system, the gel was stained for 15 min in 0.2% (wt/vol) Coomassie brilliant blue R–(wt/vol)–50% (vol/vol) methanol–10% (vol/vol) acetic acid to enable visualization of histones. Histone bands were then excised, soaked for 30 min in 6 M urea–0.37% Triton X-100–0.9 N acetic acid (pH

2.7)-5% (vol/vol) β-mercaptoethanol, placed in sample wells of a Triton X-100-acid-urea gel, and electrophoresed as described above.

To ensure that the electrophoretic migration from the acid-urea gel bands was the same as that obtained with proteins directly dissolved in electrophoresis sample buffer, the following control was performed. A 10-μl amount of acid-extractable proteins in acid-urea sample buffer was applied to an acid-urea gel and subjected to electrophoresis at 13 mA and 110 V for 20 min. After this electrophoresis, the proteins had entered the gel as a single unresolved band. This band was stained and incubated in Triton X-100-acid-urea sample buffer as described above, and it was co-electrophoresed with acid-extractable proteins dissolved directly in Triton X-100-acid-urea sample buffer. The Triton X-100-acid-urea gel patterns obtained in this way were identical (data not shown).

Fluorography and gel slice scintillation counting. Gels were prepared for fluorography by being rinsed briefly with distilled water, soaked for 30 min in 1 M sodium salicylate (pH 5.5), and again briefly rinsed with distilled water. After the gels were dried, fluorography was performed with Kodak XR-5 film at −70°C.

For scintillation counting of gel slices, protein bands were immersed in 1.5 ml of a mixture of NCS tissue solubilizer (Amersham) and water (9:1, vol/vol), heated overnight at 60°C, combined with 15 ml of toluene-Liquiflour (New England Nuclear Corp.), and counted. Values were corrected for quenching, channel cross-over, and blanks.

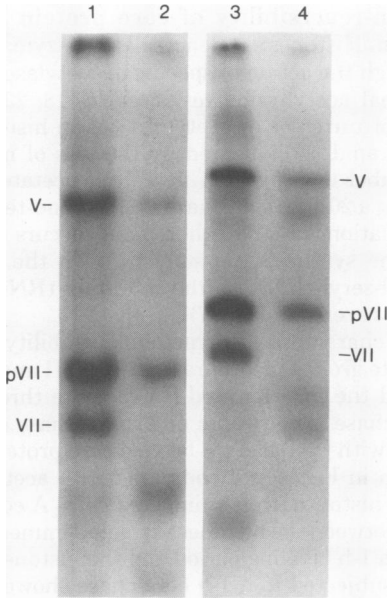

FIG. 1. *Autoradiograms of acid-extractable proteins of adenovirus-infected cell nuclei. Cells were labeled with [^{14}C]arginine (lanes 1 and 3) or [^{14}C]-sodium acetate (lanes 2 and 4) between 24 and 28 h postinfection. Acid-extractable proteins were electrophoresed on an acid-urea gel (lanes 1 and 2) and on a Triton X-100-acid-urea gel (lanes 3 and 4).*

RESULTS

Acetylation of adenovirus core proteins. To determine whether adenovirus core proteins are acetylated, infected cells were labeled for 4 h at 24 h postinfection with [^{14}C]acetate, and acid-extractable proteins were subjected to electrophoresis along with proteins concurrently labeled with [^{14}C]arginine. Figure 1 shows the autoradiographic patterns obtained. Viral proteins V, pVII, and VII incorporated the [^{14}C]-arginine label, showing that all of these proteins were synthesized during the labeling period. However, V and pVII incorporated the [^{14}C]acetate label, whereas VII did not. Uptake of the [^{14}C]acetate label into cellular histones indicated that these proteins continued to undergo acetylation during this phase of infection although the absence of [^{14}C]arginine incorporation into histones showed that histone synthesis had stopped or was greatly reduced.

Labeling periods of 0.5 and 4 h produced qualitatively identical results, although the amount of label incorporated increased with the length of the labeling period (data not shown). We did not detect [^{14}C]acetate incorporation into core protein VII even after overexposure of the autoradiogram, indicating that the acetate incorporated into pVII was removed before or during processing into core protein VII.

Triton X-100 seems to bind to proteins in proportion to their hydrophobicity or alpha-helix content and diminishes their electrophoretic mobility accordingly (Zweidler and Cohen, Fed. Proc. **13**:926, 1972). The Triton X-100-acid-urea gel system therefore effected protein separation on the basis of charge, size, and hydrophobicity or alpha-helical content. A comparison of the behavior of the proteins acid extracted from infected cells during electrophoresis with and without Triton X-100 indicated that core protein VII was relatively less retarded than histone H1, thereby enabling resolution of VII and H1, which was not possible with the acid-urea gel system of Panyim and Chalkley (Fig. 1).

The Triton X-100-acid-urea gel system successfully resolved the mono-, di-, and triacetylated forms of histones (10) distinguished by a single charge difference (e.g., histone H4 in Fig. 1). However, only one charge species of each of the core proteins could be observed with both [^{14}C]arginine labeling and [^{14}C]acetate labeling. This pattern suggests that all protein V and protein pVII molecules are acetylated to the same extent, although the presence of different forms could be obscured by precisely compensating charge modifications.

Non-reversibility of core protein acetylation. Histones are deacetylated enzymatically through the action of specific deacetylases upon internal acetyllysine residues (16, 18, 23). The rate of turnover of acetyl groups in histones is very rapid, as indicated by the loss of most of the label incorporated in a brief acetate pulse during a 20-min cold chase (21). Amino-terminal acetylation, on the other hand, occurs during histone synthesis, possibly through the use of acetyl-seryl-tRNA as the initiating tRNA, and is not reversible (21, 27).

To characterize the metabolic stability of the acetate groups incorporated into pVII and V, we traced the incorporated [^{14}C]acetate through a cold chase. The results of a pulse-chase experiment with [^{14}C]acetate-labeled core proteins are shown in Fig. 2 and compared with acetate-labeled histones from uninfected cells. A comparison between the histones extracted immediately after a 1-h labeling period and the histones from cells subjected to a 1-h cold chase showed that most of the histone label disappeared during the cold chase, whereas the amount of label in the viral proteins remained unchanged. The [^{14}C]acetate label in proteins V and pVII remained throughout chase periods of at least 4 h (data not shown), suggesting that core protein acetylation is not reversible in the same way that histone acetylation is reversible.

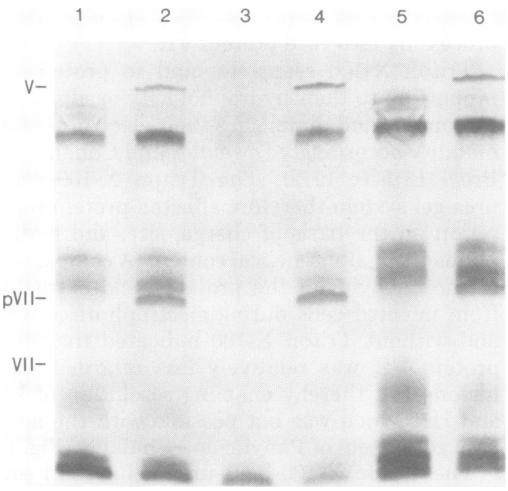

FIG. 2. *Pulse-chase of [^{14}C]acetate-labeled proteins in the presence and absence of sodium butyrate. Triton X-100–acid–urea gel autoradiogram of acid-extractable proteins from uninfected (lane 1) and adenovirus-infected cells (lane 2) labeled for 1 h with [^{14}C]acetate; proteins from uninfected (lane 3) and infected cells (lane 4) labeled for l h with [^{14}C]acetate and chased for 1 h in nonradioactive medium; proteins from uninfected (lane 5) and infected cells (lane 6) labeled for 1 h with [^{14}C]acetate and chased for 1 h in nonradioactive medium containing sodium butyrate.*

The enzymes responsible for deacetylation of internal acetyllysine residues are inhibited by butyrate treatment in vivo and in vitro, producing hyperacetylation of histones (10, 38). When the cold chase was carried out in the presence of sodium *n*-butyrate, histone deacetylation was inhibited. This resulted in a net increase in the amount of acetylation. For example, the more highly acetylated species of histone H4 predominated in the presence of butyrate. However, butyrate produced no change in either the extent of labeling of pVII and V or the number of acetylated species detectable, suggesting that inhibition of acetyllysine deacetylases had no effect on viral protein acetylation (Fig. 2 and Table 1).

Core protein VII did incorporate the [^{3}H]arginine label in the presence of butyrate (Table 1), indicating that processing of acetylated pVII into unacetylated VII did not require the action of the histone deacetylases that are susceptible to butyrate inhibition. Indeed, infectious virions are produced in nearly normal yields from butyrate-treated cells (Daniell, manuscript in preparation).

A more precise analysis of the reversibility of core protein acetylation was carried out by simultaneously labeling infected cells with [^{14}C]-acetate and [^{3}H]methionine and following both isotopes through chase periods of 1, 2, and 4 h by scintillation counting of gel slices. At all times, the ratio of ^{14}C to ^{3}H remained constant (data not shown), indicating that the acetate label was as stable as the amino acid label.

Susceptibility of adenovirus protein acetylation to cycloheximide. The acetylation of viral proteins pVII and V does not display the rapid rate of turnover or enrichment by butyrate treatment that is characteristic of the acetylation of internal lysine residues of histones. However, acetate groups are removed from pVII during the proteolytic removal of 20 amino-terminal amino acid residues in the production of core protein VII. These data suggest that the acetylation of the viral core proteins may be solely in the form of an amino-terminal acetylation that occurs during protein synthesis and remains metabolically stable thereafter. If this were the case, inhibition of protein synthesis would be expected to prevent the incorporation of [^{14}C]acetate into viral proteins, whereas the internal, postsynthetic acetylation of histones would be affected only slightly (21). By labeling infected and uninfected cells simultaneouly with [^{14}C]acetate and [^{3}H]arginine, the effect of inhibition of protein synthesis on acetate incorporation could be ascertained (Table 1). Cyclohex-

imide treatment reduced [^{3}H]arginine incorporation into adenovirus proteins V and pVII and cellular histones to less than 10% of the levels found in untreated cells. In the case of histone H1, which is known to be acetylated amino-terminally but not internally, and of viral proteins V and pVII, [^{14}C]acetate incorporation was reduced proportionately. Incorporation of [^{14}C]acetate into histone H2B, which is acetylated internally, and histone H2A, which is acetylated both amino-terminally and internally, was only minimally affected. The proportionality between protein synthesis and acetylation for adenovirus proteins pVII and V suggests that these proteins are permanently acetylated amino-terminally at the time of synthesis and do not undergo reversible internal acetylation. Amino-terminal acetylation of pVII is consistent with the finding that the amino terminus of pVII is blocked in terms of dansylation and reaction with phenylisothiocyanate (28, 42).

Effect of adenovirus infection on host histone acetylation. Histone modification through acetylation has been postulated to play a role in the assembly of newly synthesized histones onto DNA and in modulation of transcriptional activity (2, 3, 39). Because adenovirus infection inhibits host DNA and histone syntheses and represses host transcriptional activity, it was of interest to determine whether adenovirus infection altered normal patterns of histone acetylation. A comparison of the amounts of [^{14}C]acetate incorporation into the histones of infected and uninfected cells during a 2-h labeling period revealed no substantial difference in the steady-state levels of acetylation for histones H2B and H3 (Table 2). The levels of acetylation of H2A and H4 were somewhat reduced. The lower level of H2A acetylation observed in infected cells may have been a result of adenovirus-induced repression of histone synthesis, as H2A acetylation was also reduced by cycloheximide. However, the reduction of H4 acetylation in infected cells, appears to be greater than that observed in cycloheximide-treated cells.

A comparison of the [^{14}C]acetate-labeled histones from infected and uninfected cells after a cold chase (Fig. 2) showed that more label remained in the histones from infected cells. This suggests that rates of deacetylation are lower in adenovirus-infected cells.

TABLE 1. *Effects of cycloheximide and sodium butyrate on incorporation of [^{14}C]acetate and [^{3}H]arginine into viral proteins and histones*[a]

Protein	Radioactivity (cpm) in:				Cycloheximide: % of untreated prepn		Radioactivity (cpm) in butyrate-treated prepn		Butyrate: % of untreated prepn	
	Untreated prepn		Cycloheximide-treated prepn							
	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C
V	2,280	240	120	26	5.3	11	2,160	220	95	92
pVII	4,610	430	230	60	5.0	14	4,510	470	98	110
VII	260	ND[b]	30	ND	12		250	ND	96	
H1	350	130	16	25	4.6	19	340	160	97	120
H2A	1,160	470	87	250	7.5	53	960	1,890	83	400
H2B	1,530	940	62	1,130	4.0	120	1,150	2,720	75	290

[a] Cells were labeled for 2 h at 27 h postinfection. Protein bands were excised from an acid-urea gel for counting, except in the case of protein VII, which was isolated from a Triton X-100–acid–urea gel.

[b] ND, Not detectable.

TABLE 2. *Effect of adenovirus type 5 infection on host histone acetylation*[a]

Histone	Radioactivity (cpm) in uninfected control		% of control					
			Adenovirus type 5 infected		Cycloheximide treated		Adenovirus type 5 infected and cycloheximide treated	
	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C
H2A	1,160	470	23	57	7	53	2	58
H2B	1,530	940	20	90	4	120	2	105
H3	850	960	23	97	3	84	3	98
H4	1,400	2,340	36	44	6	108	4	54

[a] Cells were labeled for 2 h at 27 h postinfection with [^{14}C]acetate or [^{3}H]arginine. Protein bands were excised from acid-urea gels for counting.

DISCUSSION

Histones are among the most highly conserved proteins in terms of primary structure and among the most highly modified proteins. In many cases, histone modifications, acetylation in particular, have been correlated with changes in transcriptional activity, chromatin assembly, and chromatin condensation (19). Cellular enzymes which are normally active upon a histone-containing chromatin substrate are diverted to viral expression in the course of the adenovirus replication cycle. However, the substrate for viral transcription and replication presented to the host synthetic machinery appears to differ from normal eucaryotic chromatin. It was our intention to determine whether acetylation plays a role in adenovirus expression.

The precursor of the major core protein of adenovirus (protein pVII) and the minor adenovirus core protein (protein V) appear to be acetylated, as indicated by the incorporation of radiolabeled acetate into these proteins. The processed form of the major core protein (protein VII) is not acetylated.

Two types of histone acetylation are known to occur. Reversible acetylation of the epsilon amino group of internal lysine residues occurs in core histones H2A, H2B, H3, and H4, neutralizing the basic charge of these residues. Charge modification through lysine acetylation occurs within the basic portion of the histone polypeptide that is believed to be involved in electrostatic interactions between histones and the negatively charged phosphate backbone of DNA. Lysine acetyl groups undergo rapid turnover. Therefore, this type of acetylation and deacetylation may serve to modulate the affinity of histones for DNA and may be involved in the transient alterations of chromatin structure that accompany transcription and replication (3).

Our observations indicate that the conversion of acetylated pVII to unacetylated VII occurs through a mechanism different than that involved in the interconversion of acetylated and unacetylated forms of histones. Sodium butyrate, which inhibits histone deacetylation, fails to inhibit the production of VII from pVII. Furthermore, the stability of [^{14}C]acetate label in viral proteins pVII and V through cold chase periods of up to 4 h is in contrast to the rapid metabolic turnover of acetyllysine in cellular histones. These observations and the existence of only one acetylated species of pVII and V suggest a constant unmodulated level of acetylation intrinsic to these proteins.

As has been suggested by Sung et al. (28, 42), the hydrophobic portion of pVII may be functionally analogous to the acetylated amino-terminal regions of histones. Proteolytic cleavage of the precursor portion of pVII may substitute for a reversible deacetylation mechanism for condensation and packaging of the viral DNA. If pVII participates in the structure of the late transcription complex, its primary structure may be sufficient to ensure the recognition of the viral chromatin as "active" chromatin even in the absence of internal acetylation.

The absence of reversible acetylation of viral histone-like proteins may reflect the basic differences in replicative strategy between cells and viruses. Viral replication proceeds in a linear fashion from disassembly of parental virus and maximally efficient replication of virion components to assembly of progeny virus from newly synthesized proteins and nucleic acids. In contrast to the role of histones in chromosome condensation during mitosis or transient transcriptional activity, virus structural proteins participate in a specific replicative phase only once. Therefore, a reversible mechanism for "inactivation" and chromatin condensation may be unnecessary.

Nonreversible amino-terminal acetylation has been shown to be characteristic of histones H1, H2a, and H4 (18), as well as approximately 50 other proteins (7), including the capsid proteins of polyoma virus (17), the large T and small t antigens of simian virus 40 (35), and the hexon protein of the adenovirus capsid (22). This modification of the amino terminus renders a protein nonreactive to protein-sequencing reagents that require a free amino group (36). The precursor of the major adenovirus core protein (42), as well as other adenovirus structural proteins, has this characteristic, but the processed form of the major core protein does not (26). In view of our findings regarding acetate incorporation into viral proteins pVII and V, its metabolic stability, its dependence on protein synthesis, and its lack of responsiveness to butyrate treatment, it appears likely that the blocked amino termini of these proteins are a result of amino-terminal acetylation.

The functional significance of amino-terminal acetylation remains to be clearly defined. This amino-terminal modification affords protection from aminopeptidases and may contribute to the metabolic stability of the proteins (7). The presence of an acetate group in an amide linkage serves to neutralize a positive charge that would otherwise be present at the amino-terminal residue. The resulting hydrophobicity may be significant in terms of membrane interactions or interactions with hydrophobic domains of other proteins. Cleavage of the hydrophobic portion of pVII may then be required for proper folding of the viral DNA during encapsidation (6, 28, 42).

LITERATURE CITED

1. **Alfageme, C. R., A. Zweidler, A. Mahowald, and L. Cohen.** 1974. Histones of *Drosophila* embryos. J. Biol. Chem. **249**:3729–3736.
2. **Allfrey, V. G.** 1970. Changes in chromosomal proteins at times of gene activation. Fed. Proc. **29**:1447–1459.
3. **Allfrey, V. G., R. Faulkner, and A. E. Mirsky.** 1964. Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis. Proc. Natl. Acad. Sci. U.S.A. **51**:786–794.
4. **Amin, M., A. Mirza, and J. Weber.** 1977. Genetic analysis of adenovirus type 2. VII. Cleavage modified affinity for DNA of internal virion proteins. Virology **80**:83–97.
5. **Amin, M., A. Mirza, and J. Weber.** 1979. Uncoating of adenovirus type 2. J. Virol. **30**:462–471.
6. **Anderson, C. W., P. R. Baum, and R. F. Gesteland.** 1973. Processing of adenovirus 2-induced proteins. J. Virol. **12**:241–252.
7. **Bloemendal, H.** 1977. The vertebrate eye lens. Science **197**:127–138.
8. **Candido, E. P. M., and G. H. Dixon.** 1972. Trout testis cells. III. Acetylation of histones in different cell types from developing trout testis. J. Biol. Chem. **247**:5506–5510.
9. **Corden, J., H. M. Engelking, and G. D. Pearson.** 1976. Chromatin-like organization of the adenovirus chromosome. Proc. Natl. Acad. Sci. U.S.A. **73**:401–404.
10. **Cousens, L. S., D. Gallwitz, and B. M. Alberts.** 1979. Different accessibilities in chromatin to histone acetylase. J. Biol. Chem. **254**:1716–1723.
11. **Edvardsson, B., E. Everitt, H. Jörnvall, L. Prage, and L. Philipson.** 1976. Intermediates in adenovirus assembly. J. Virol. **19**:533–547.
12. **Edvardsson, B., S. Ustacelebi, J. Williams, and L. Philipson.** 1978. Assembly intermediates among adenovirus type 5 temperature-sensitive mutants. J. Virol. **25**:641–651.
13. **Everitt, E., S. A. Meador, and A. S. Levine.** 1977. Synthesis and processing of the precursor to the major core protein of adenovirus type 2. J. Virol. **21**:199–214.
14. **Felsenfeld, G.** 1978. Chromatin—review article. Nature (London) **271**:115–122.
15. **Flint, J.** 1977. The topography and transcription of the adenovirus genome. Cell **10**:153–166.
16. **Gallwitz, D.** 1971. Organ specifity of histone acetyltransferases. FEBS Lett. **13**:306–308.
17. **Hewick, R. M., A. Mellor, A. E. Smith, and M. D. Waterfield.** 1980. Partial amino-terminal sequences of the polyoma nonhistone proteins VP1, VP2, and VP3 synthesized in vitro. J. Virol. **33**:631–636.
18. **Inoue, A., and D. Fujimoto.** 1970. Histone deacetylase from calf thymus. Biochim. Biophys. Acta **220**:307–316.
19. **Isenberg, I.** 1979. Histones. Annu. Rev. Biochem. **48**: 159–191.
20. **Ishibashi, M., and J. V. Maizel, Jr.** 1974. The polypeptides of adenovirus. V. Young virions, structural intermediates between top components and aged virions. Virology **57**:409- 424.
21. **Jackson, V., A. Shires, R. Chalkley, and D. K. Granner.** 1975. Studies on highly metabolically active acetylation and phosphorylation of histones. J. Biol. Chem. **250**:4856–4863.
22. **Jörnvall, H., H. Ohlsson, and L. Philipson.** 1974. An acetylated N-terminus of adenovirus type 2 hexon protein. Biochem. Biophys. Res. Commun. **56**:304–310.
23. **Kikuchi, H., and D. Fujimoto.** 1973. Multiplicity of histone deacetylase from calf thymus. FEBS Lett. **29**: 280–282.
24. **Kit, G., and E. Daniell.** 1978. Adenovirus core protein synthesis in the absence of viral DNA synthesis late in infection. J. Virol. **27**:74–80.
25. **Laver, W. G.** 1970. Isolation of an arginine-rich protein from particles of adenovirus type 2. Virology **41**:488–500.
26. **Laver, W. G., J. R. Suriano, and M. Green.** 1967. Adenovirus proteins. II. N-terminal amino acid analysis. J. Virol. **1**:723–728.
27. **Liew, C. C., G. W. Haslett, and V. G. Allfrey.** 1970. N-acetyl-seryl-tRNA and polypeptide chain initiation during histone biosynthesis. Nature (London) **226**:414–417.
28. **Lischwe, M. A., and M. T. Sung.** 1977. A histone-like protein from adenovirus chromatin. Nature (London) **267**:552–554.
29. **Lonberg-Holm, K., and L. Philipson.** 1969. Early events of virus-cell interaction in an adenovirus system. J. Virol. **4**:323–338.
30. **Maizel, J. V., Jr., D. O. White, and M. D. Scharff.** 1968. The polypeptides of adenovirus. I. The evidence for multiple protein components in the virion and a comparison of types 2, 7A, and 12. Virology **36**:115–125.
31. **Maizel, J. V., Jr., D. O. White, and M. D. Scharff.** 1968. The polypeptides of adenovirus. II. Soluble proteins, cores, top components, and the structure of the virion. Virology **36**:125–131.
32. **Miller, O. L., and L. Hodge.** 1975. Visualization of a unique adenovirus DNA-protein complex. J. Cell Biol. **67**:289a.
33. **Nadler, K. D.** 1976. Histone acetylation during meiosis in *Lilium* microsporocites. Exp. Cell Res. **101**:283–292.
34. **Panyim, S., and R. Chalkley.** 1969. High resolution acrylamide gel electrophoresis of histones. Arch. Biochem. Biophys. **130**:337–346.
35. **Paucha, E., A. Mellor, R. Harvey, A. E. Smith, R. M. Hewick, and M. D. Waterfield.** 1978. Large and small tumor antigens from simian virus 40 have identical amino termini mapping at 0.65 map units. Proc. Natl. Acad. Sci. U.S.A. **75**:2165–2169.
36. **Phillips, D. M. P., and E. W. Johns.** 1965. A fractionation of the histones of group F2a from calf thymus. Biochem. J. **94**:127–130.
37. **Prage, L., and U. Pettersson.** 1971. Structural proteins of adenoviruses. VII. Purification and properties of an arginine-rich core protein from adenovirus type 2 and type 3. Virology **45**:364–373.
38. **Riggs, M. G., R. G. Whittacker, J. R. Neuman, and V. M. Ingram.** 1977. n-Butyrate causes histone modification in HeLa and Friend erythroleukaemia cells. Nature (London) **268**:462–464.
39. **Ruiz-Carrillo, A., L. J. Wangh, and V. G. Allfrey.** 1975. Processing of newly synthesized histone molecules. Science **190**:117-128.
40. **Russell, W. C., K. McIntosh, and J. J. Skehel.** 1971. The preparation and properties of adenovirus cores. J. Gen. Virol. **11**:35–46.
41. **Sergeant, A., M. A. Tigges, and H. J. Raskas.** 1979. Nucleosome-like structural subunits of intranuclear parental adenovirus type 2 DNA. J. Virol. **29**:888–898.
42. **Sung, M. T., M. A. Lischwe, J. C. Richards, and K. Hosokawa.** 1977. Adenovirus chromatin. I. Isolation and characterization of the major core protein VII and precursor pro-VII. J. Biol. Chem. **252**:4981–4987.
43. **White, D. O., M. D. Scharff, and J. V. Maizel, Jr.** 1969. The polypeptides of adenovirus. III. Synthesis in infected cells. Virology **3**:395–406.
44. **Winnacker, E.-L.** 1978. Adenovirus DNA: structure and function of a novel replicon. Cell **14**:761–773.