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Adenovirus Core Protein Synthesis in the Absence of Viral DNA Synthesis Late in Infection

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The acid extraction of the adenovirus type 5 core proteins V, VII, and pVII (the precursor to VII) from infected cells and the subsequent electrophoresis on a 15% acrylamide-2.5 M urea-0.9 N acetic acid (pH 2.7) gel, revealed that peptide VII has a similar electrophoretic mobility to that of histone H1. The core proteins, which are coded by late adenovirus mRNA, continued to be synthesized late in infection when viral DNA synthesis was inhibited either by cytosine arabinoside in wild-type infections or by shifting adenovirus H5 ts125-infected cells to the nonpermissive temperature (40°C). Only the initiation, not the continuation, of viral DNA replication was essential for core protein synthesis. The synthesis of viral core proteins continued for over 8 h after the cessation of DNA synthesis. This was in contrast to the rapid shutdown of cellular histone synthesis in the absence of cellular DNA synthesis.

The DNA of eucaryotic cells is complexed with a globular cluster of eight histones (two each of H2a, H2b, H3, and H4) into nucleohistone complexes called nucleosomes (28). These structures are the means by which vast lengths of cell DNA are condensed and packaged into the nucleus. Nucleosomes are also found associated with the DNA of simian virus 40 and polyoma virus forming minichromosomes (8). However, in virions from adenovirus types 2, 3, 5 (Ad5), 7, and 12, there are no histones (18, 19). Instead, the viral DNA cores contain two viral specific, arginine-rich proteins: core-1 (45,000 molecular weight) and core-2 (18,000 molecular weight) or peptides V and VII, respectively (16, 18, 19, 23, 25). These core proteins take the place of histones in condensing adenovirus DNA during packaging.

Histones are only synthesized in the S phase of the cell cycle, i.e., during the period of DNA synthesis. The tight coupling of DNA synthesis and histone synthesis has been shown in various cell types (3, 5, 30), and in productive infections with simian virus 40 and polyoma virus where viral DNA replication is associated with the S phase of the cell cycle and histone synthesis (8, 29). If cycloheximide is used to inhibit histone synthesis, then cell and/or papova viral DNA synthesis is inhibited (3, 13, 15, 30). Conversely, when cytosine arabinoside (araC) or fluorodeoxyuridine is used to inhibit cell or polyoma virus DNA synthesis, histone synthesis is immediately shut off (5, 26).

Stein et al. (27) present evidence that the coupling of histone synthesis and DNA synthesis in HeLa S_3 cells is not controlled at the transcriptional level. The amount of histone mRNA sequences in nuclei of S-phase cells treated with araC or hydroxyurea is the same as that in untreated S-phase cells. In addition, although treatment with araC or hydroxyurea results in a loss of histone mRNA from the polysomes, there is an elevated level of histone mRNA in the nonpolysomal cytoplasmic fraction. Stein suggests that this histone mRNA is in an untranslatable form and concludes that the coupling of DNA and histone synthesis is a post-transcriptional process (27).

Upon infection with adenovirus, host cell DNA and histone synthesis are inhibited (11). Furthermore, when adenovirus-infected cells are treated with cycloheximide, viral DNA synthesis is not inhibited if cycloheximide is added after the initiation of viral DNA synthesis (12). Thus, it is possible that the synthesis of core proteins is independent of viral DNA synthesis late in infection.

Carter and Ginsberg have shown when araC is added to Ad5-infected cells after the initiation of DNA synthesis, DNA synthesis is subsequently inhibited, but late mRNA continues to be made. Late mRNA synthesis also continues when H5 ts125-infected cells are shifted to the nonpermissive temperature after DNA synthesis has been initiated (6). Among the late proteins coded for in this message are the two histone-like core proteins: V and pVII (the precursor to VII) (9).

In this communication, we have tried to determine if adenovirus DNA synthesis and core

protein synthesis exhibit a tight coupling similar to that observed between cell and/or papova viral DNA synthesis and histone synthesis.

MATERIALS AND METHODS

Cells, virus, and viral infections. HeLa cells were cultured in minimum essential medium (Earle salts) supplemented with 5% fetal calf serum. Ad5 was grown on HeLa monolayers. The infected cells were washed with phosphate-buffered saline and subjected to sonic treatment. The resulting sonically treated lysate was titered and used for infections. H5 ts125 was a kind gift of H. Ginsberg. Viral infections were carried out on confluent HeLa monolayers at a multiplicity of 60 PFU/cell. Virus was absorbed for 1 h at 37°C. After absorption, the infected cells were fed with minimum essential medium supplemented with 5% dialyzed fetal calf serum.

Radioactive labeling and drugs. Cells were labeled with 0.5 μCi of [^{14}C]arginine per ml (298 mCi/mmol) in minimum essential medium supplemented with 5% dialyzed fetal calf serum and harvested after labeling. AraC was used at either 2, 20, or 200 μg/ml and was added 30 min before labeling.

Acid-soluble protein extractions. Acid-soluble proteins were extracted by using a modification of the histone extraction procedure of Hodge and Scharff (11). Cells were scraped from 100-mm dishes with a rubber policeman and then washed twice with phosphate-buffered saline. All subsequent steps were carried out at 4°C. Nuclei were isolated by treating the cells with 0.5% Triton X-100–0.5% Nonidet P-40–0.2 mM phenylmethylsulfonylfluoride for 15 min and subsequently pelleted at 1,500 × *g* for 5 min. Globins were extracted by resuspending the nuclei in 10 mM Tris-hydrochloride (pH 7.4)–0.14 M NaCl–1.5 mM $MgCl_2$–0.2 mM phenylmethylsulfonylfluoride for 30 min. Nuclei were again pelleted at 1,500 × *g* for 5 min. The acid-soluble proteins were then extracted from the nuclei by resuspending them in 0.2 M H_2SO_4–0.2 mM phenylmethylsulfonylfluoride for 30 min. Finally, the nuclei were pelleted at 1,500 × *g* for 5 min, and the acid-soluble proteins were precipitated from the supernatant in 9 volumes of acetone overnight. The resulting pellet was dissolved in 50 μl of 15% sucrose–2.5 M urea–0.9 N acetic acid (pH 2.7).

Gel electrophoresis and autoradiography. A 15% acrylamide–2.5 M urea–0.9 N acetic acid (pH 2.7) slab gel (0.15 by 10 by 14 cm) was prepared by the method of Panyin and Chalkley (22). The composition of the gel was as follows: 15 ml of freshly prepared 0.2% ammonium persulfate (wt/vol) in 4.0 M urea was added to 3.0 ml of 43.2% glacial acetic acid (vol/vol), 4.0% *N,N,N',N'*-tetramethylenediamine (wt/vol) in water, and 6.0 ml of 60% acrylamide (wt/vol), 0.4% *N,N'*-bisacrylamide (wt/vol) in water. A 2.5 M urea–0.9 N acetic acid (pH 2.7) buffer was used for electrophoresis. Gels were subjected to pre-electrophoresis at 20 mA until the methyl green tracking dye ran out of the gel (about 6 h). A 10-μl amount of each sample was then subjected to electrophoresis for 9 h at a constant current of 12 mA, 115 V. After electrophoresis, gels were stained with 0.5% amido black 10B–7% acetic acid for 30 min and destained overnight in 35% ethanol–7% acetic acid (20). Gels were dried down and autoradiographed using Kodak No-Screen Ns-5T X-ray film.

RESULTS

Identification of adenovirus core proteins. To make a comparison between the control of histone synthesis and the control of adenovirus core protein synthesis, it was necessary to find a system whereby both groups of proteins could be visualized at the same time. Both histones and adenovirus core proteins are highly basic; thus, the high-resolution gel system for basic proteins developed by Panyin and Chalkley (22) was well suited for this purpose.

An autoradiograph of the five histones from uninfected HeLa cells is shown in Fig. 1a. Cells were labeled for 8 h with [^{14}C]arginine, and the

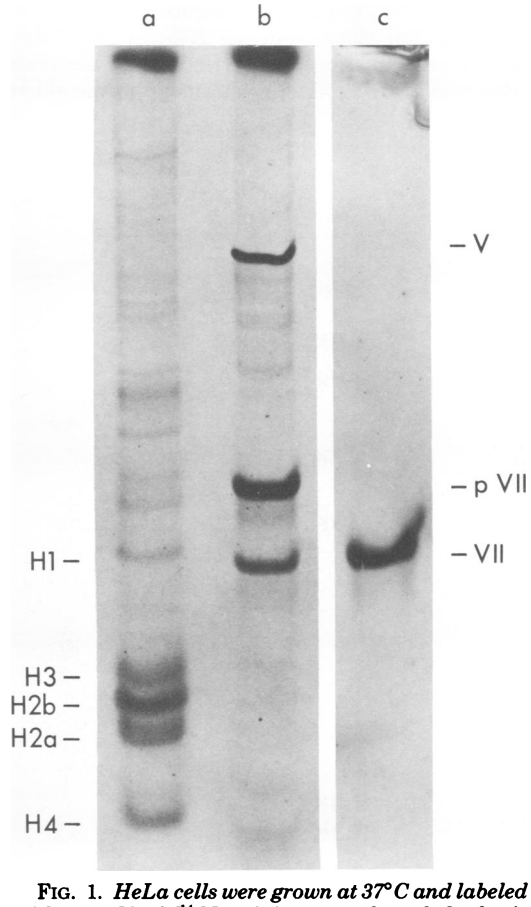

FIG. 1. *HeLa cells were grown at 37°C and labeled with 0.5 μCi of [^{14}C]arginine per ml, and the basic proteins were extracted and subjected to electrophoresis for 9 h at 12 mA, 115 V. (a) and (b) are autoradiographs which have been exposed for 12 days, whereas (c) is stained with amido black 10B (see text). (a) Uninfected cells labeled for 8 h, (b) Ad5-infected cells labeled 24 to 32 h p.i., (c) purified VII.*

basic proteins were extracted and subjected to electrophoresis (see above). When cells were infected with Ad5 and labeled with [^{14}C]arginine from 24 to 32 h postinfection (p.i.), three main bands appeared on the autoradiograph. They have been designated V, pVII, and VII (Fig. 1b). Histones do not appear in Fig. 1b because histone synthesis is turned off late in adenovirus infections (11).

To confirm the identification of major core protein VII, purified peptide VII (a gift of U. Pettersson) was subjected to co-electrophoresis with uninfected and Ad5-infected cells and stained with amido black 10B (Fig. 1c). As in the infected-cell preparation, peptide VII ran just ahead of histone H1.

In vivo (1) and in vitro (2, 7, 21) translation experiments have shown that the processing of pVII into VII requires the removal of about 15 to 20 amino acids from the *N* terminal. Pulse-chase experiments were carried out to illustrate this precursor-product relationship in the above gel system (data not shown). Our results showed that we could chase the band labeled pVII into VII. Thus, it appears that H1 and VII, which both have a molecular weight about 18,000, have similar electrophoretic mobilities.

Peptide V was identified by running a portion of the [^{14}C]arginine-labeled acid-extractable proteins from infected cells on a 12.5% polyacrylamide-sodium dodecyl sulfate gel (Fig. 2c). Again three main viral-specific bands appear on the gel. A comparison of these three bands with [^{14}C]arginine-labeled proteins from purified Ad5 virion (Fig. 2d) and known molecular weight standards (Fig. 2a and e) shows that these bands correspond to peptide V (molecular weight, 45,000), peptide pVII (molecular weight, 20,000), and peptide VII (molecular weight, 18,000). Due to their high basicity, peptides pVII and VII run more slowly than β-lactoglobulin (molecular weight, 18,400) on SDS gels.

Ad5 core protein synthesis and viral DNA synthesis in the presence of araC.

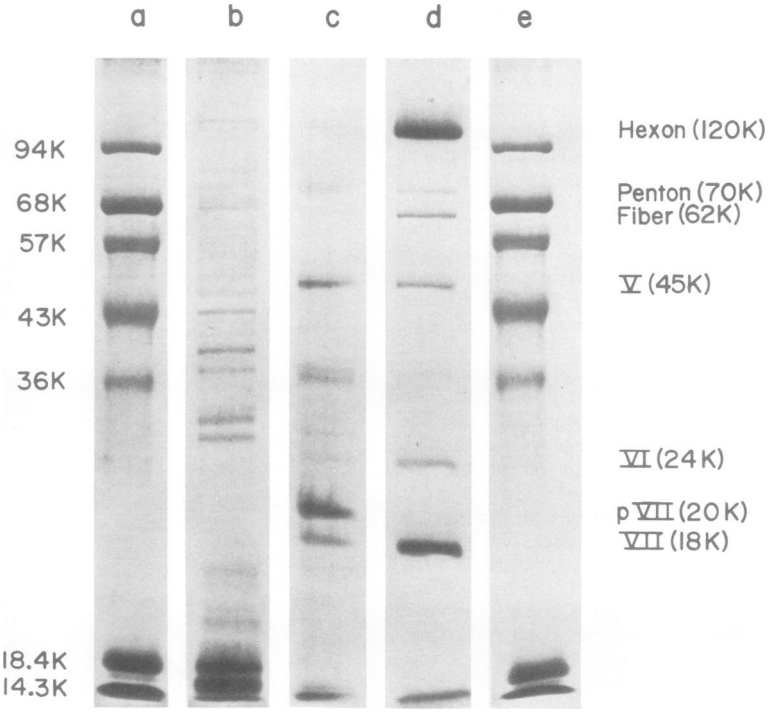

FIG. 2. *Molecular weight determination of basic proteins extracted from uninfected cells (not labeled), Ad5-infected cells labeled with 0.5 μCi of [^{14}C]arginine per ml, and Ad5 virion proteins labeled with [^{14}C]arginine. A 12.5% polyacrylamide–0.1% sodium dodecyl sulfate–0.325% N,N'-bisacrylamide gel (1.5 mm by 10 cm by 14 cm) subjected to electrophoresis at 110 V for 5 h. (a), (b), and (c) have been stained with 0.2% Coomassie brilliant blue–10% acetic acid–50% methanol and destained with 7.5% acetic acid and 10% methanol. (c) and (d) have been autoradiographed for 12 days. (a) Molecular weight standards: phosphorylase A, bovine serum albumin, pyruvate kinase, ovalbumin, lactate dehydrogenase, β-lactoglobulin, and lysozyme. (b) Uninfected cell. (c) Ad5-infected cells labeled 24 to 32 h p.i. (d) Ad5 virion proteins. (e) Molecular weight standards as in (a).*

When araC was added to uninfected cells, histone synthesis was inhibited. Figure 3 shows an autoradiograph of the basic proteins extracted from uninfected cells that had been labeled for 8 h with [^{14}C]arginine in the absence (Fig. 3a) and presence (Fig. 3b) of 20 μg of araC per ml. However, when Ad5-infected cells were treated with from 2 to 200 μg of araC per ml and labeled with [^{14}C]arginine from 24 to 32 h p.i., core proteins continued to be synthesized (Fig. 3c, d, e, and f). In infected cells treated with 20 μg of araC per ml, DNA synthesis measured by the incorporation of [^{3}H]deoxythymidine into trichloroacetic acid-precipitable counts was over 90% inhibited (data not shown).

To show that the initiation of viral DNA synthesis is essential before uncoupling of core protein synthesis from viral DNA synthesis takes place, infected cells were treated with araC and labeled with [^{14}C]arginine from 8 to 24 h p.i. The labeling period extended from the end of the early phase through the late phase of infection. DNA synthesis had not begun by 8 h p.i. in our system and was completely blocked at this time by araC treatment. Figure 4a shows that in the absence of araC, core proteins are made during this period. On the other hand, when infected cells were treated with araC, there was no detectable core protein synthesis during the period (Fig. 4b). It should be noted that although histones were not made late in infection (Fig. 1b), they were synthesized early in infection (Fig. 4a) and could still be turned off with araC (Fig. 4b).

The synthesis of adenovirus core proteins in the presence of araC extends beyond 8 h after the addition of araC. This was shown by adding araC to Ad5-infected cells 16 h p.i. and then labeling with [^{14}C]arginine in the presence of araC from 24 to 32 h p.i. (Fig. 4d). It is apparent that less core protein is synthesized in the presence of araC if the drug is added at 16 h p.i. We believe that this is due to the smaller amount of viral DNA available for transcription when viral DNA synthesis is inhibited this early in infection.

Ad5 core protein synthesis and viral DNA synthesis in H5 ts125-infected cells.

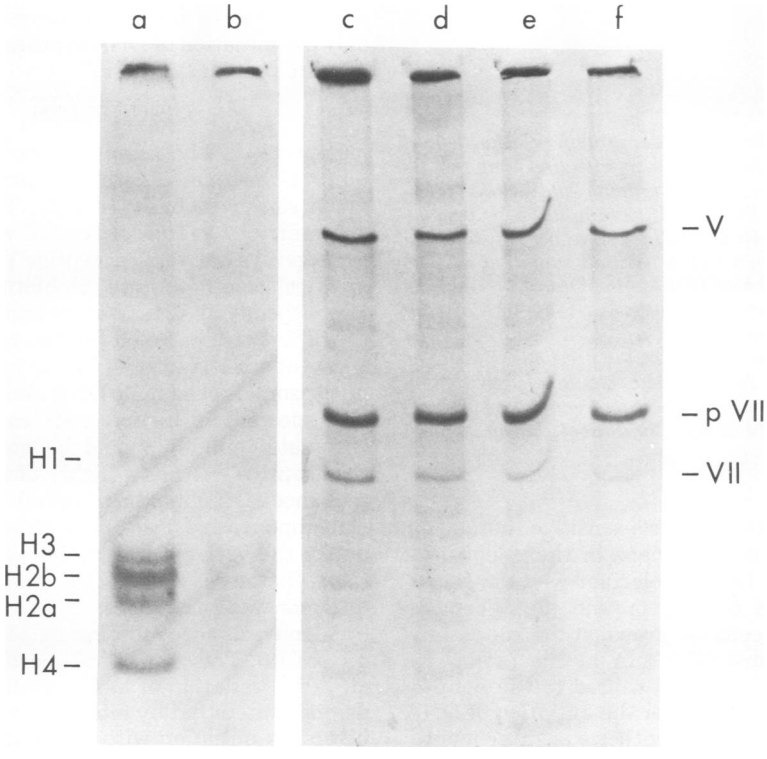

FIG. 3. *Uninfected and Ad5-infected HeLa cells treated with and without araC. Uninfected cells were labeled for 8 h and Ad5-infected cells grown at 37°C were labeled from 24 to 32 h p.i. with 0.5 μCi of [^{14}C]arginine per ml, and the basic proteins were extracted, subjected to electrophoresis for 9 h at 12 mA, 115 V, and autoradiographed for 12 days (see text). (a) Uninfected cells, (b) uninfected cells plus 20 μg of AraC per ml, (c) Ad5-infected cells, (d) Ad5-infected cells plus 2 μg of AraC per ml, (e) Ad5-infected cells plus 20 μg of AraC per ml, (f) Ad5-infected cells plus 200 μg of araC per ml.*

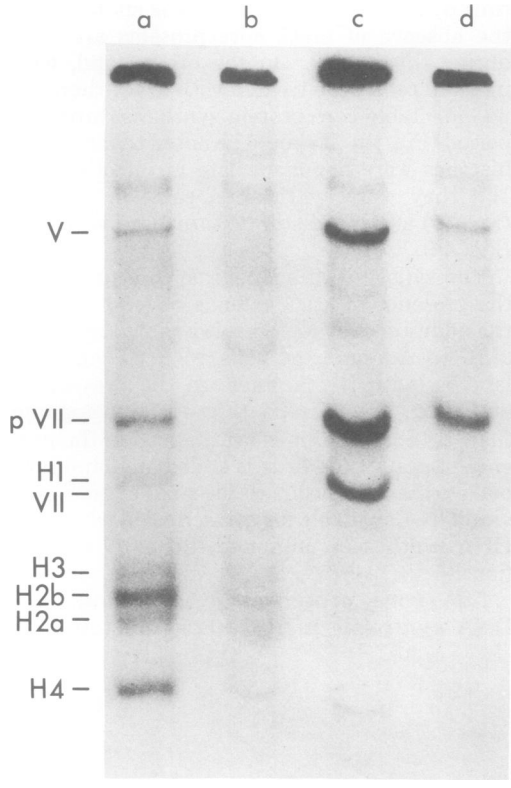

FIG. 4. *Coupling of core protein synthesis and viral DNA synthesis early in infection and the extended synthesis of core proteins late during infection. Infected cells at 37°C were labeled for various times with 0.5 μCi of [^{14}C]arginine per ml, and the basic proteins were extracted and subjected to electrophoresis for 9 h at 12 mA, 115 V. Autoradiographs were exposed for 5 days (see text). (a) Ad5-infected cells labeled from 8 to 24 h p.i., (b) Ad5-infected cells labeled from 8 to 24 h p.i. in the presence of 20 μg of araC per ml, (c) Ad5-infected cells labeled 24 to 32 h p.i., (d) Ad5-infected cells treated with 20 μg of araC per ml from 16 to 32 h p.i. and labeled from 24 to 32 h p.i.*

H5 ts125 is a temperature-sensitive mutant for DNA replication and maps in the region of a viral-specific, 72,000-molecular-weight, single-stranded DNA binding protein (9). When H5 ts125-infected cells are grown at the nonpermissive temperature (40°C) or at the permissive temperature (32°C) and shifted to the nonpermissive temperature after the initiation of DNA synthesis, viral DNA synthesis is subsequently inhibited (6).

Adenovirus core proteins are synthesized 32 to 40 h p.i. in H5 ts125-infected cells at the permissive temperature but are not synthesized during the same period when infected cells are grown at the nonpermissive temperature (Fig. 5c and d). However, in wild-type Ad5 infections, core proteins are made at the nonpermissive temperature (Fig. 5b). Thus, at least the initiation of viral DNA synthesis is essential for core protein synthesis.

When H5 ts125-infected cells were grown for 16 h at 32°C, then shifted up to 40°C and labeled with [^{14}C]arginine from 16 to 40 h p.i., no core proteins were synthesized (Fig. 5f). A shift to the nonpermissive temperature at 16 h after infection results in the production of only early RNA and no late RNA (4). However, if H5 ts125-infected cells remained at the permissive temperature and were labeled for the same period, core proteins were synthesized (Fig. 5e). Hence, a shift up to the nonpermissive temperature early in the course of infection inhibits core protein synthesis.

On the other hand, core proteins are synthesized after a shift up to the nonpermissive temperature late in infection. In addition, core proteins continue to be synthesized beyond 8 h after shifting up. H5 ts125-infected cells were grown for 24 h at 32°C, then shifted to 40°C and labeled 24 to 32 h p.i. and 32 to 40 h p.i. with [^{14}C]-arginine (Fig. 5g and h). These results show that only the initiation of DNA synthesis is necessary for core protein synthesis.

DISCUSSION

Peptide VII has a similar electrophoretic mobility as histone H1, whereas peptide V has a much slower mobility (Fig. 1). This finding is contradictory to that of Russell who, using the same gel system, reports peptide V and VII have identical electrophoretic mobilities as H1 and H3, respectively (24). We are unable to explain the differences between the two results.

Histone H1 is located in the region between nucleosomes on cellular DNA and is believed to be important in higher order condensation of chromatin (10). It should be noted, however, that peptide VII has a highly basic *N*-terminal sequence (DNA binding region) similar to H4 (a component of the nucleosome) (17) and is unlike the variable *N*-terminal sequence of H1 (10). Therefore, H1 and VII probably act in a different way to condense DNA.

Adenovirus core proteins are coded for in late mRNA (9). This is the viral-specific RNA made after the initiation of DNA synthesis. We have demonstrated that by inhibiting DNA synthesis before its initiation with either the addition of araC into the medium or by growing H5 ts125 at the nonpermissive temperature, core proteins are not synthesized (Fig. 4a and b and 5e and f). However, if DNA synthesis is inhibited after it has been initiated, core proteins continue to be synthesized (Fig. 3 and 5g). In addition, because

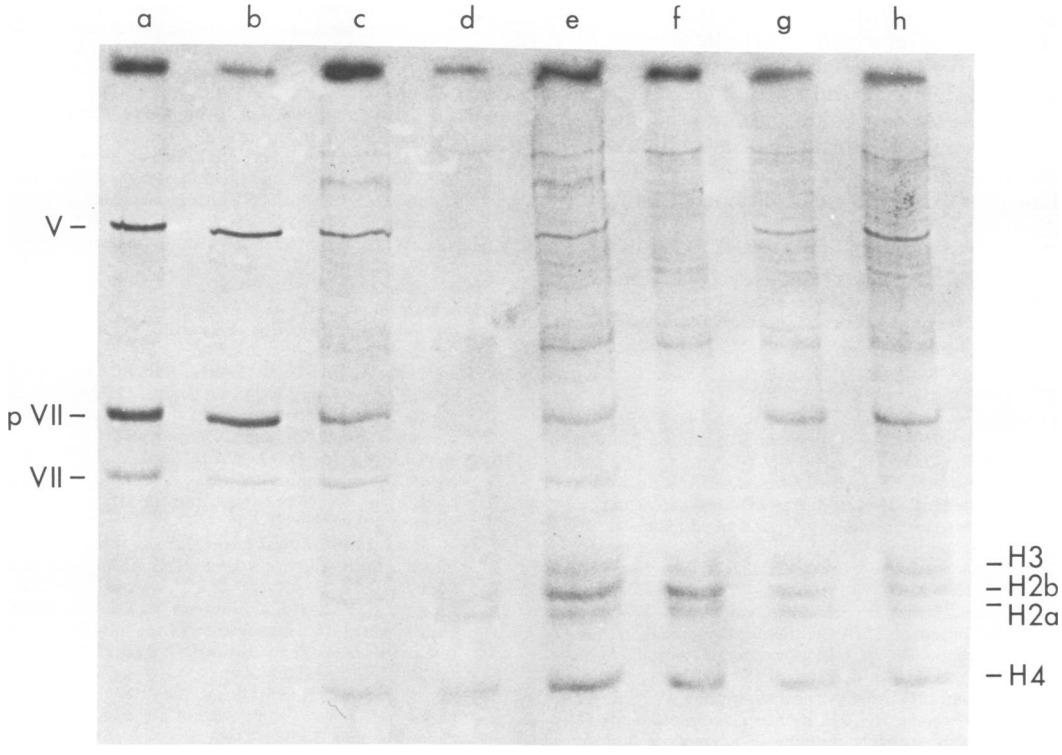

FIG. 5. *Uncoupling of core protein synthesis and DNA synthesis in H5 ts125-infected cells. Cells were labeled with 0.5 μCi of [^{14}C]arginine per ml, and the basic proteins were extracted, subjected to electrophoresis for 9 h at 12 mA, 115 V, and autoradiographed for 12 days (see text). (a) Ad5-infected cells grown at 37°C and labeled 24 to 32 h p.i., (b) Ad5-infected cells grown at 40°C and labeled 24 to 32 h p.i., (c) H5 ts125-infected cells grown at 32°C and labeled 32 to 40 h p.i., (d) H5 ts125-infected cells grown at 40°C and labeled 32 to 40 h p.i., (e) H5 ts125-infected cells grown at 32°C and labeled from 16 to 40 h p.i. at 32°C, (f) H5 ts125-infected cells grown at 32°C from 0 to 16 h p.i., then shifted up to 40°C and labeled 16 to 40 h p.i., (g) H5 ts125-infected cells grown at 32°C from 0 to 24 h p.i., then shifted up to 40°C and labeled 24 to 32 h p.i., (h) H5 ts125-infected cells grown at 32°C from 0 to 24 h p.i., then shifted up to 40°C at 24 h p.i. and labeled 32 to 40 h p.i.*

core protein synthesis continues beyond 8 h after the inhibition of viral DNA synthesis, it appears that the uncoupling of viral DNA synthesis and core protein synthesis is permanent (Fig. 4d and 5h).

The synthesis of adenovirus core proteins uncoupled from viral DNA synthesis occurs only after the initiation of viral DNA synthesis. This mechanism is in contrast to the tight coupling of cell and/or papova viral DNA synthesis and histone synthesis. In these cases, histone gene expression is dependent upon continual DNA synthesis.

Late adenovirus mRNA has a polyadenylic acid tail (8), whereas histone mRNA does not (14). It is possible that the absence of a polyadenylic acid tail in histone mRNA facilitates the coupling of DNA and histone synthesis at a posttranscriptional level, as suggested by Stein et al. (27). It is apparent from our results that in adenoviruses there is a different relationship between viral DNA synthesis and expression of core proteins.

The majority of late mRNA sequences are transcribed from the *r* strand. It is believed that mRNA species are cleaved from a large transcription product of this strand. These mRNA species are then modified and transported to the cytoplasm for translation (9). Therefore, it is probable that the transcription of late mRNA species is controlled as a unit, i.e., late genes are coordinately expressed.

It has been proposed that the control of late gene expression is dependent upon the accumulation of an early gene product(s) that modifies the form II RNA polymerase of the cell, which allows the transcription of late mRNA (9). Because the presence of araC during the course of infection does not inhibit the expression of early gene products (4), but does inhibit core protein synthesis, this model seems unlikely.

In the light of our results, a more attractive

model might be that there is a limited amount of host repressor which acts at a late gene control site. During the viral DNA synthesis, repressor sites eventually exceed the number of repressor molecules. At this point, inhibition of late gene expression is overcome. Thus, even if DNA synthesis is inhibited late in adenovirus infection, late gene products can continue to be synthesized.

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LITERATURE CITED

1. **Anderson, C. W., P. R. Baum, and R. F. Gesteland.** 1973. Processing of adenovirus 2-induced proteins. J. Virol. **12**:241-252.
2. **Anderson, C. W., J. B. Lewis, J. F. Atkins, and R. F. Gesteland.** 1974. Cell-free synthesis of adenovirus 2 proteins programmed by fractionated messenger RNA: a comparison of polypeptide products and messenger RNA lengths. Proc. Natl. Acad. Sci. U.S.A. **71**:2756-2760.
3. **Bennet, L. L., D. Smithers, and C. T. Ward.** 1964. Inhibition of DNA synthesis in mammalian cells by actidione. Biochim. Biophys. Acta **87**:60-69.
4. **Berget, S. M., S. J. Flint, J. F. Williams, and P. A. Sharp.** 1976. Adenovirus transcription. IV. Synthesis of viral-specific RNA in human cells infected with temperature-sensitive mutants of adenovirus 5. J. Virol. **19**:879-889.
5. **Borun, T. W., M. D. Scharff, and E. Robbins.** 1967. Rapidly labeled, polyribosome-associated RNA having the properties of histone message. Proc. Natl. Acad. Sci. U.S.A. **58**:1977-1983.
6. **Carter, T. H., and H. S. Ginsberg.** 1976. Viral transcription in KB cells infected by temperature-sensitive "early" mutants of adenovirus type 5. J. Virol. **18**:156-166.
7. **Eron, L., H. Westphal, and R. Callahan.** 1974. In vitro synthesis of adenovirus core proteins. J. Virol. **14**:375-383.
8. **Fareed, G. C., and D. Davoli.** 1977. Molecular biology of papovaviruses. Annu. Rev. Biochem. **46**:471-552.
9. **Flint, J.** 1977. The topography and transcription of adenovirus genome. Cell **10**:153-166.
10. **Hartman, P. G., G. E. Chapman, T. Moss, and E. M. Bradbury.** 1977. Studies on the role and mode of operation of the very lysine-rich histone H1 in eukaryote chromatin. Eur. J. Biochem. **77**:45-52.
11. **Hodge, L. D. and M. D. Scharff.** 1967. Effect of adenovirus on host cell DNA synthesis in synchronized cells. Virology **37**:554-564.
12. **Horwitz, M. S., C. Brayton, and S. G. Baum.** 1973. Synthesis of type 2 adenovirus DNA in the presence of cycloheximide. J. Virol. **11**:544-551.
13. **Kang, H. S., T. B. Eshbach, D. A. White, and A. J. Levine.** 1971. Deoxyribonucleic acid replication in simian virus 40-infected cells. IV. Two different requirements for protein synthesis during simian virus 40 deoxyribonucleic acid replication. J. Virol. **7**:112-120.
14. **Kedes, L. H.** 1976. Histone messengers and histone genes. Cell **8**:321-332.
15. **Kit, S., T. Kurimura, R. A. de Torres, and D. R. Dubbs.** 1969. Simian virus 40 deoxyribonucleic acid replication. I. Effect of cycloheximide on the replication of SV40 deoxyribonucleic acid in monkey kidney cells and in heterokaryons of SV40-transformed and susceptible cells. J. Virol. **3**:25-32.
16. **Laver, W. G.** 1970. Isolation of an arginine-rich protein from particles of adenovirus type 2. Virology **41**:488-500.
17. **Lischwe, M. A., and M. T. Sung.** 1977. A histone-like protein from adenovirus chromatin. Nature (London) **267**:552-554.
18. **Maizel, J. V., Jr., D. O. White, and M. D. Scharff.** 1968. The polypeptides of adenovirus. I. Evidence for multiple protein components in the virion and a comparison of type 2, 7A, and 12. Virology **36**:115-125.
19. **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.
20. **Moll, R. and E. Wintersberger.** 1976. Synthesis of yeast histones in the cell cycle. Proc. Natl. Acad. Sci. U.S.A. **73**:1863-1867.
21. **Öberg, B., J. Saborio, T. Persson, E. Everitt, and L. Phillipson.** 1975. Identification of the in vitro translation products of adenovirus mRNA by immunoprecipitation. J. Virol. **15**:199-207.
22. **Panyin, S., and R. Chalkley.** 1969. High resolution acrylamide gel electrophoresis of histones. Arch. Biochem. Biophys. **130**:337-346.
23. **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.
24. **Russell, W. C.** 1971. The acid-soluble components of adenovirus and of adenovirus infected cells. J. Gen. Virol. **11**:65-69.
25. **Russell, W. C., K. McIntosh, and J. J. Skehel.** 1971. The preparation and properties of adenovirus cores. J. Gen. Virol. **11**:35-46.
26. **Seehafer, J. G., and P. Weil.** 1974. Synthesis of polyoma virus structural polypeptides in mouse kidney cells. Virology **58**:75-85.
27. **Stein, G., J. Stein, E. Sheperd, W. Park, and I. Phillips.** 1977. Evidence that the coupling of histone gene expression and DNA synthesis in HeLa S_3 cells is not mediated at the transcriptional level. Biochem. Biophys. Res. Commun. **77**:245-252.
28. **Thomas, J. O., and R. D. Kornberg.** 1975. An octomer of histones in chromatin and free in solution. Proc. Natl. Acad. Sci. U.S.A. **72**:2626-2630.
29. **Winocour, E., and E. Robbins.** 1970. Histone synthesis in polyoma and SV40-infected cells. Virology **40**:307-315.
30. **Young, C. W.** 1966. Inhibitory effects of acetoxycycloheximide, puromycin, and pactamycin upon synthesis of protein and DNA in asynchronous populations of HeLa cells. Mol. Pharmacol. **2**:50-55.