HISTONE SYNTHESIS IS NOT COUPLED TO THE REPLICATION OF ADENOVIRUS DNA*

Gary Tallman[+], James E. Akers[†], Byron T. Burlingham[†], and Gerald R. Reeck[+]

[†]Division of Biology and [+]Department of Biochemistry
Kansas State University, Manhattan, Kansas 66506

and

[†]Department of Microbiology, East Carolina University
Greenville, North Carolina 27834

Received October 19,1977

SUMMARY

Histone synthesis decreases approximately in parallel with the decrease in cellular DNA synthesis when KB cell monolayers are productively infected with adenovirus type 2 and does not occur in coordination with the later surge of viral DNA synthesis. The synthesis of histones is not, therefore, required for all replicative DNA synthesis in the nuclei of mammalian cells.

INTRODUCTION

Histones, the major structural proteins of eukaryotic chromosomes, are synthesized concomitantly with DNA in the S phase of the cell life cycle (1). Inhibition of DNA synthesis with drugs results in a cessation of histone synthesis (1). Introduction of inhibitors of protein synthesis into the culture medium of mammalian cells immediately reduces the rate of DNA synthesis about fivefold and halts DNA synthesis altogether in about one hour (2). These observations support an obligatory coupling of the synthesis of nuclear DNA and histones (3). Recent evidence has extended this concept to include nuclear synthesis of the exogenous DNA of simian virus 40 (4).

In the first few hours after infection of cells with adenoviruses, there is a gradual decline in cellular DNA synthesis that is followed by replication of viral DNA (5-7). This sequence of events provides the opportunity to ask whether histone synthesis accompanies synthesis of adenoviral

*Reprints may be obtained from either Dr. Burlingham (East Carolina University) or Dr. Reeck.

ISSN 0006-291X

DNA in the host cells' nuclei. We have examined this by measuring the incorporation of both [^{14}C]-deoxythymidine into DNA and [^{3}H]-lysine into histones at several times after the infection of KB cells with adenovirus type 2.

MATERIALS AND METHODS

Cells and virus

KB cells (Flow Laboratories) were grown in monolayer culture in Eagle's minimal essential medium supplemented with 5% calf serum. Adenovirus type 2, obtained from the American Type Culture Collection, was propagated in KB cell cultures. To purify adenovirus, we modified the procedure of Doerfler (8) to include 3 CsCl density gradient centrifugations, as well as zonal centrifugation.

Synchronous infection of KB cells and pulse labeling

Each monolayer of KB cells (1-5 X 10^7 cells) was infected with adenovirus at a multiplicity of 50-100 plaque forming units of purified virus per cell. The inoculum was diluted in 5 ml of phosphate-buffered saline containing 0.2% bovine serum albumin and adsorbed for 2 hr at 4°. The cells were then washed three times with phosphate-buffered saline at 4° to remove unadsorbed virus. After 2, 6, 12, 18, or 24 hr of incubation at 37°, growth medium was replaced with minimal essential medium deficient in lysine and supplemented with 5% calf serum, 10 µCi/ml of [^{3}H]-lysine (59 Ci/mmole, New England Nuclear) and 5 nCi/ml of [^{14}C]-deoxythymidine (57 mCi/mmole, New England Nuclear). After a 30 min labeling period, the cells were released by a 10 min incubation in 25 ml of 1 mM EDTA/10 mM Tris·Cl (pH 7.4) and then collected by centrifugation.

Preparation of KB cell nuclei

Cells collected from each monolayer were suspended in 2 ml of 10 mM NaCl/1.5 mM $MgCl_2$/10 mM Tris·Cl (pH 7.4) containing 1 mM phenylmethane sulfonylfluoride, swelled on ice for 10 min, and homogenized with 10 strokes of a Potter-Elvehjem homogenizer. Crude nuclei were then collected by centrifugation at 1000g for 10 min and suspended in 2 ml of 10 mM NaCl/1.5 mM $MgCl_2$/10 mM Tris·Cl (pH 7.4) and 0.3 ml of a mixed detergent solution that contained 3% sodium deoxycholate/7% Tween 40 (9). The detergent-treated nuclei were collected by centrifugation at 2000g for 10 min and suspended in 2 ml of 10 mM NaCl/1.5 mM $MgCl_2$/10 mM Tris·Cl (pH 7.4). A small portion of each nuclei preparation was stained with toluidine blue and counted using a hemacytometer. The purified nuclei were centrifuged, suspended in 2 ml of 1 mM phenylmethane sulfonylfluoride/0.1 mM EDTA (pH 7.0), sonicated with four 30 sec bursts of an Artek Dismembrator at maximum power, and incubated at 0° for 15 min.

Extraction of acid soluble nuclear proteins

A standard procedure for acid extraction of histones (10) was applied to the nuclear lysates. 0.4 ml of cold 2 M H_2SO_4 was added slowly, with stirring, to each 2 ml suspension. After stirring for 2 hr at 4°, DNA and acid insoluble proteins were removed by centrifugation at 15,000g for 30 min at 4°. The precipitate was solubilized in 1 ml of Protosol (New England Nuclear). A 100 µl aliquot was added to 10 ml of Bray's scintillation fluid (11) and the amount of radioactivity determined using a liquid scintillation spectrometer. 10 ml of cold 95% ethanol was added to each supernatant and the samples stored at -20° for 24 hr (10). The precipitated proteins were collected by centrifugation at 15,000g for 30 min at 4° and dissolved in 0.5 ml of 1 mM phenylmethane

sulfonylfluoride/1 M Tris·Cl (pH 7.5). A 100 µl portion was added to 10 ml of Bray's scintillation fluid and the amount of tritium determined.

Gel electrophoresis and analysis

The remainder of the solubilized protein was brought to 1% sodium dodecyl sulfate, immersed in a boiling water bath for 5 min, dialyzed against 1% sodium dodecyl sulfate/1% 2-mercaptoethanol/0.1% bromphenol blue/10% glycerol/0.03 M Tris-phosphate (pH 6.8), and analyzed by discontinuous polyacrylamide gel electrophoresis (15% acrylamide:0.2% bis-acrylamide) essentially as described by Laemmli (12). After electrophoresis, the gels were severed at the tracking dye and either stained to visualize protein bands or sliced for counting. Gels were stained for 15 min with 1% amido black in 7% acetic acid and destained in 7% acetic acid/40% ethanol in the presence of Dowex 1 beads. Unstained gels were fixed in 50% trichloroacetic acid for 1 hr at 4° and sliced into 1 mm sections. Gel slices were solubilized by incubation in scintillation vials overnight at 37° in 0.3 ml of 30% hydrogen peroxide. 10 ml of Bray's scintillation fluid was added to each vial and the amount of tritium determined.

RESULTS

Figure 1 shows the incorporation of [^{14}C]-deoxythymidine into DNA (-●-) and of [^{3}H]-lysine into acid-soluble, ethanol-precipitable nuclear proteins (-○-) and into the four core histones, H2A, H2B, H3, and H4 (-□-). The deoxythymidine incorporation data are consistent with the previously documented decline of cellular DNA synthesis in the first few hours after infection with adenoviruses and the initiation of viral DNA synthesis at 10-12 hours after infection (5-7).

The incorporation of [^{3}H]-lysine into acid-soluble, ethanol-precipitable nuclear proteins parallels the deoxythymidine incorporation into DNA until 12 hours after infection but does not subsequently increase along with DNA synthesis. Furthermore, polyacrylamide gel electrophoresis revealed that from 12 to 24 hours after infection much of the lysine incorporation was not into histones (Figure 2). The amount of [^{3}H]-lysine incorporated into the core histones could be unambiguously determined because of their characteristic, high mobilities (Figure 3). At each time studied, radioactivity was also present in the position where histone H1 migrated, but the amount of incorporation into H1 was difficult to determine at 18 and 24 hours because of the presence of other large peaks of radioactivity in the same region. The incorporation of [^{3}H]-lysine into the core histones clearly decreases during

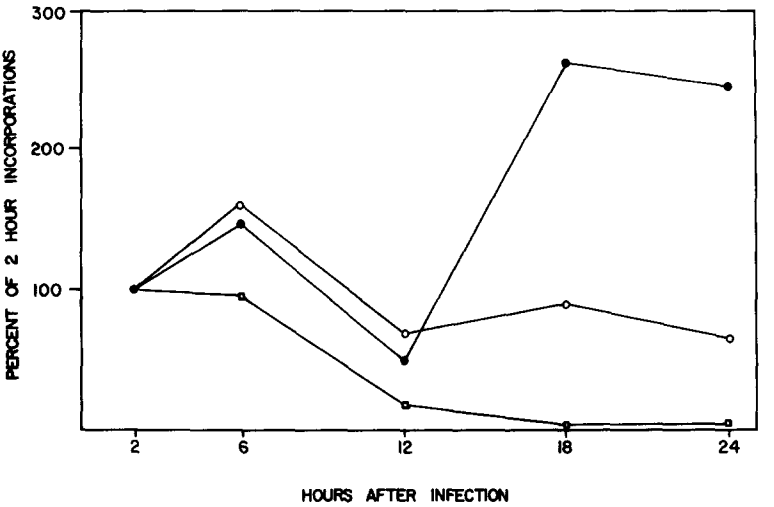

FIGURE 1. Incorporation of [^{14}C]-deoxythymidine into DNA (-●-), [^{3}H]-lysine into acid-soluble, ethanol-precipitable nuclear proteins (-O-), and [^{3}H]-lysine into the core histones, H2A, H2B, H3, and H4 (-□-) at several times after infection of KB cells with adenovirus type 2. Infected cells were exposed to the radioactive compounds for 30 min at the indicated times, and incorporations determined as described in the text. The amounts of [^{3}H]-lysine in the core histones were calculated by multiplying the incorporation into acid-soluble, ethanol-precipitable nuclear proteins by the percentage of this incorporation that was in the core histones, as determined by gel electrophoresis (see Figure 2). Incorporations of the radioactive compounds into the relevant macromolecules were normalized to 10^7 nuclei and percentages calculated relative to the incorporations observed at 2 hr after infection. The 2 hr incorporations, as measured in 100 µl samples, were: 91,356 counts per minute of [^{3}H]-lysine and 716 counts per minute of [^{14}C]-deoxythymidine into acid-soluble, ethanol-precipitable nuclear proteins and DNA, respectively.

the late stages of infection (Figure 1) when adenovirus DNA is being synthesized in large amounts; the ratio of [^{3}H]-lysine incorporation into the core histones to the [^{14}C]-deoxythymidine incorporation into DNA is approximately 50-fold lower at 18 hours after infection that at 2 hours after infection.

At 12, 18, and 24 hours after infection, one of the major bands of radioactivity observed on polyacrylamide gels migrates between histones H1 and H3 with a mobility that coincides with that of a major band visualized by staining (Figure 3). This band has a slightly lower mobility than the major core protein (polypeptide VII) from mature adenovirions and is most likely the precursor of polypeptide VII (13). Other major, unidentified peaks of

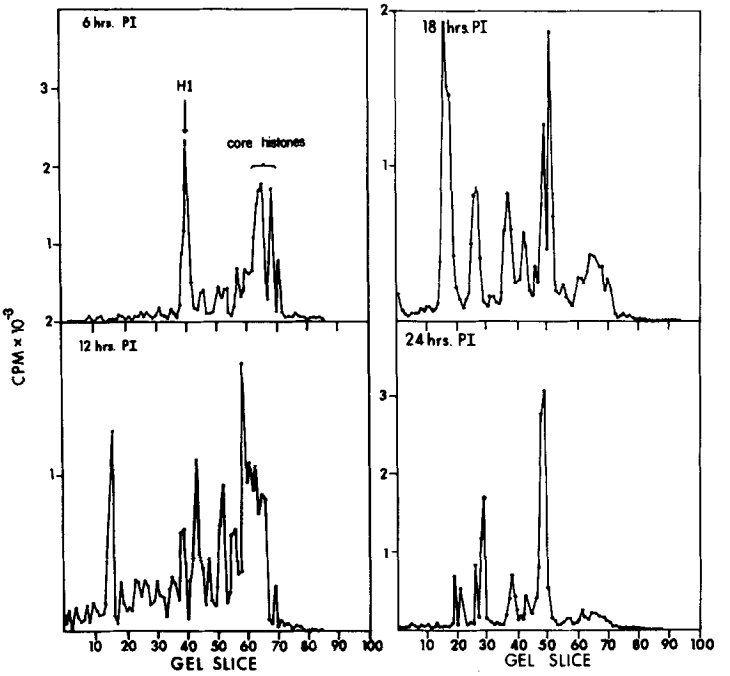

FIGURE 2. Sodium dodecyl sulfate/polyacrylamide gel electrophoresis of [^{3}H]-lysine labeled, acid-soluble, ethanol-precipitable nuclear proteins from KB cells infected with adenovirus type 2. The mobilities of the histones were established from gels run in parallel on the same samples and stained for protein (see Figure 3).

radioactivity are present as well. For the most part, these do not correspond in mobility to proteins found in mature virions.

DISCUSSION

To the extent that our pulse-labeling experiments reflect rates of macromolecular synthesis, our results suggest that the rate of histone synthesis decreases after infection of KB cells with adenovirus type 2, at least roughly parallel to the decrease in the rate of cellular DNA synthesis. Russell (14) has previously suggested that histone synthesis declines after infection of human embryonic kidney cells with adenovirus type 5, but the interpretation of his data is limited by the comigration of two viral core proteins with histones H1 and H3 in the electrophoresis system he used and

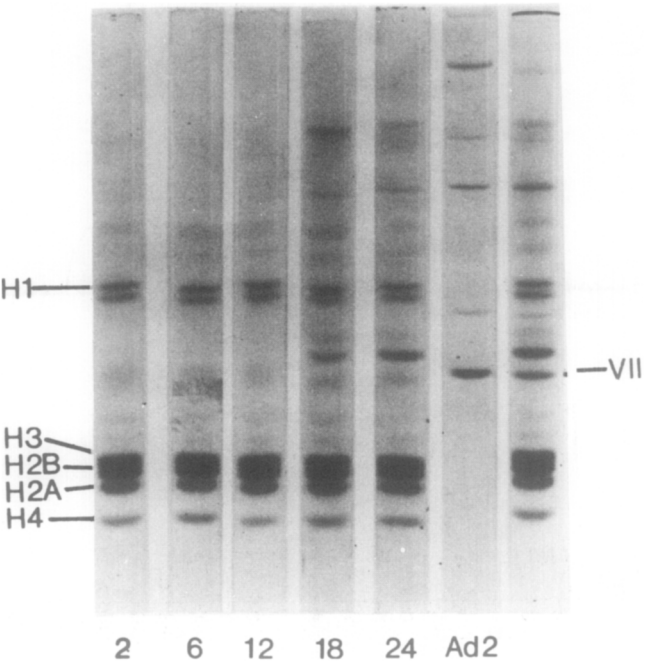

FIGURE 3. Sodium dodecyl sulfate/polyacrylamide gel electrophoresis of, from left to right: acid-soluble, ethanol-precipitable nuclear proteins from KB cells at 2, 6, 12, 18, and 24 hr after infection; adenovirus type 2 (Ad2); a mixture of the samples for gels 5 and 6. The gels were stained with amido black for photography. The histones and virion polypeptide VII (last two gels) are identified.

by an absence of data on DNA synthesis.

Histone synthesis is stimulated after infection of cells with papovaviruses (15-17), which, in contrast to adenoviruses, possess histones as structural components of virions (18,19). Tan (4) has recently reported that 30-60 hours after the infection of monkey cells with simian virus 40, histones are synthesized in coordination with viral DNA replication. Our results indicate that histone synthesis does not occur in coordination with replication of adenovirus DNA. This implies that histone biosynthesis is not an obligatory correlate of nuclear DNA synthesis. Raydt et al. (20) have reached a similar conclusion from their studies on the action of 2,3,5-tris-ethyleneiminobenzoquinone, a drug that inhibits histone synthesis in cultured

mouse cells but does not interfere with DNA synthesis. The absence of histone synthesis during the replication of adenovirus DNA implies that studies on the mechanism of adenovirus DNA synthesis may not be relevant in all respects to understanding the mechanism of cellular DNA synthesis.

The lack of coordinated synthesis of histones and adenovirus DNA does not exclude the possibility that histones might form complexes with adenovirus DNA. Recent studies by Seale (21) and Weintraub (22) indicate that DNA synthesized in the presence of inhibitors of protein synthesis is complexed with histones, but at a lower histone/DNA mass ratio than DNA synthesized in untreated cells. This suggests that histones that were complexed with previously synthesized DNA migrate to newly synthesized DNA. Migration of histones during replication of cellular DNA has been directly documented by Seale (23) and by Jackson et al. (24) in studies of the histones bound to newly synthesized DNA. Based on these studies, it seems possible that histones complexed with cellular DNA might redistribute to adenovirus DNA. Such a migration may be inevitable unless specifically prevented by a viral mechanism. If histones were transferred to viral DNA, their absence in virions might be due to selective proteolysis of histones on the viral DNA (our gels indicate that there is not a generalized destruction of histones in the nuclei of infected cells) or to displacement by the major core protein (polypeptide VII) or its precursor.

ACKNOWLEDGMENTS

We thank Bruce C. Black for supplying some of the cells and virus used in this study and Mark H. Spurrier for his participation in working out the labeling conditions. This work was supported by grants from the Anna Fuller Fund and the National Institutes of Health (CA-17782) to GRR, a grant from the Mid-America Cancer Center Program (NCI-CA-15080-03) to BTB, and by the Kansas Agricultural Experiment Station. Contribution 78-55-j, Department of Biochemistry and Division of Biology.

REFERENCES

1. Robbins, E., and Borun, T. W. (1967) Proc. Nat. Acad. Sci. USA 57, 409-416
2. Seale, R. L., and Simpson, R. T. (1975) J. Mol. Biol. 94, 479-501.
3. Stein, G., Stein, J., Shephard, E., Park, W., and Phillips, I. (1977) Biochem. Biophys. Res. Commun. 77, 245-252.

4. Tan, K. B. (1977) Proc. Nat. Acad. Sci. USA 74, 2805-2809.
5. Pina, M., and Green, M. (1969) Virology 38, 573-586.
6. Burger, H., and Doerfler, W. (1974) J. Virology 13, 975-992.
7. Ginsberg, H. S., Bello, L. J., and Levine, A. J. (1967) in The Molecular Biology of Viruses (J. S. Colter and W. Paranchych, Eds.) Academic Press, New York, pp. 547-572.
8. Doerfler, W. (1969) Virology 38, 587-606.
9. Penman, S. (1969) in Fundamental Techniques in Virology (K. Habel and N. P. Salzman, Eds.) Academic Press, New York, pp. 35-48.
10. Bonner, J., et al. (1968) Methods in Enzymology 12B, 20-21.
11. Bray, G. A. (1960) Anal. Biochem. 1, 279-285.
12. Laemmli, U. K. (1970) Nature 227, 680-685.
13. Anderson, C. W., Baum, P. R., and Gesteland, R. F. (1973) J. Virology 12, 241-252.
14. Russell, W. C. (1971) J. Gen. Virol. 11, 65-69.
15. Shimono, H., and Kaplan, A. S. (1969) Virology 37, 690-694.
16. Hancock, R., and Weil, R. (1969) Proc. Nat. Acad Sci. USA 63, 1144-1150.
17. Winocour, E., and Robbins, E. (1970) Virology 40, 307-315.
18. Lake, R. S., Barban, S., and Salzman, N. P. (1973) Biochem. Biophys. Res. Commun. 54, 640-647.
19. McMillen, J., and Consigli, R. A. (1974) J. Virology 14, 1326-1336.
20. Raydt, G., Holzweber, F., Puschendorf, B., and Grunicke, H. (1977) FEBS Lett. 74, 111-114.
21. Seale, R. L. (1976) Cell 9, 423-429.
22. Weintraub, H. (1976) Cell 9, 419-422.
23. Seale, R. L. (1976) Proc. Nat. Acad. Sci. USA 73, 2270-2274.
24. Jackson, V., Granner, D. K., and Chalkley, R. (1976) Proc. Nat. Acad. Sci. USA 73, 2266-2269.