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Histone synthesis during infection of monkey kidney cells with Simian Virus 40

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ABSTRACT

The synthesis of histones during lytic infection of BSC-1 (African Green Monkey kidney) cells with SV40 has been investigated. The synthesis of all five classes of histones was stimulated, and all classes appeared to be stimulated to the same extent. The increase in rate of histone synthesis in response to SV40 infection was detectable several hours before SV40 DNA synthesis was measureable, and the rate of histone synthesis decreased at a time when SV40 DNA synthesis was occuring at a maximal or relatively high rate. In addition, the changes in rates of histone synthesis did not correlate well with the rates of host DNA synthesis during infection. Thus it appears that DNA synthesis and histone synthesis may not be strictly coupled in SV40 infected cells.

INTRODUCTION

Purified Simian Virus (SV40) virions contain several proteins (1, 2). Three of these are virus coded, and are designated VP1, VP2 and VP3. There are also four smaller basic proteins of cellular origin. These have been shown to be the four small histones H3, H2A, H2B and H4 (3) which form the nucleosome core in cellular chromatin. They appear to play the same role in SV40 (4), and the SV40-DNA-histone complex is often called a "minichromosome". The fifth major cellular histone, H1, is not detected in SV40 virions, but it has been reported that SV40 minichromosomes isolated directly from infected cells do contain H1 (5).

In uninfected growing cells, the synthesis of histone mRNA and of histones themselves is tightly coupled to cellular DNA synthesis (6, 7). Winocour and Robbins showed, using mouse cells infected lytically with polyoma virus or abortively with SV40, that increased histone synthesis is observed at the time of induction of cellular DNA synthesis (8). They did not study rates of nistone synthesis at different times after infection nor attempt to correlate histone synthesis either with cellular DNA synthesis or with viral DNA synthesis. The difficulty with making such a correlation is that infection with SV40 stimulates cellular DNA synthesis in most cell

lines (9), as does addition of fresh serum (10). Viral DNA synthesis may therefore represent only a small fraction of the total DNA synthesis occuring in the cell, with the contribution of viral DNA synthesis to rate of histone synthesis (assuming such a relationship) being correspondingly small. Recently, Liberti et al correlated the rate of histone mRNA synthesis in SV40 infected CV-1 cells with the synthesis of both SV40 DNA and cellular DNA, thus suggesting some relation between SV40 DNA synthesis and that of histone synthesis in CV-1 cells. They also showed that the messages could be translated in a cell free system to produce all histone classes including H1 (11).

We present here studies on the rates of synthesis of viral and cellular DNA and of histones at various times after SV40 infection. We have used the BSC-1 monkey kidney cell line in which the induction of cellular DNA synthesis by SV40 infection is less than in other commonly used permissive cell lines (e.g. primary AGMK cells or CV-1 cells) (9).

MATERIALS AND METHODS

Virus and cells. SV40 strain 777 and the established line of African Green Monkey kidney cells, BSC-1, were used throughout. Preparation of plaque-purified viral stocks and growth of cells was as described previously (12).

Infection of cells. Medium was removed from confluent monolayers of BSC-1 cells (about 3×10^6 cells/plate) and virus (10-40 PFU/cell) was added in 1 ml of Eagle's medium supplemented with 2% fetal bovine serum (2E2). 1 ml of 2E2 alone was added to mock infected cells. Virus was allowed to adsorb for 2 hours at 37°C. The medium containing unadsorbed virus was then removed, 10 ml of 2E2 was added to each plate and incubation was continued for the times shown in the text. Time zero was taken to be the time of addition of the viral stocks.

Labeling of cells. Medium was removed from the cells and 5 ml of Eagle's medium containing 0.1 the customary concentration of lysine, 1% dialyzed fetal bovine serum and either [^{3}H]lysine (N.E.N. 10 µCi/ml) or [^{3}H]thymidine (Schwarz/Mann, 1 or 10 µCi/ml) were added. Incubation was continued for 1 hour at 37° in all cases.

Isolation of DNA. At the end of the incubation period, cells labeled with [^{3}H]thymidine were treated by the method of Hirt (13). The supernatant fluid containing mainly viral DNA and the pellet containing mainly cellular DNA were prepared for counting as described before (12).

Extraction of proteins.

a) Acid extraction. At the end of the labeling period, medium containing

[^{3}H]lysine was removed from the plates. An ice-cold solution (1.5 ml) containing 50 mM sodium bisulphite, 1 mM phenylmethylsulphonyl fluoride (PMSF) and 2 mM EDTA (all to reduce proteolytic activity) was added and the cells were scraped into a tube with a rubber policeman. The plates were washed with a further 1.5 ml of the same solution and the suspensions were pooled. One-tenth volume of 4.4 $\underline{N}$ H_2SO_4 was added and the sample was sonicated for 3 min at full strength in a pre-cooled Raytheon sonifier. The samples were shaken for 2 hours at 4° and then centrifuged for 30 min at 17000 rpm in a Beckman JA-20 rotor (about 35000 x g). The supernatant solutions were pipetted off and the acid soluble proteins were precipitated with 5 volumes of ice-cold ethanol and stored at -20° for at least 20 hours. The solutions were centrifuged for 30 min at 17000 rpm and the precipitates were then wasned twice with ice-cold ethanol and once with acetone and then dried under vacuum at 4°.

b) Detergent extraction. After the labeling period medium was removed from the plates and 0.5 ml of polyacrylamide stacking gel buffer (14) containing, in addition to the usual components, 1% sodium dodecyl sulfate (SDS), 8 M urea, 1% 2-mercaptoethanol and 1 mM PMSF was added per plate. The lysate was scraped off into a tube with a rubber policeman.

SDS-polyacrylamide gel electrophoresis. Electrophoresis was performed on slab gels using a 4% acrylamide stacking gel - 15% separating gel system (15) or a 3%/18% modification thereof (14). Acid extracted samples were prepared for electrophoresis by dissolving in 400 µl of stacking gel buffer containing in addition 1% SDS, 8 M urea and 1% 2-mercaptoethanol. The samples were then heated at 100° for 3 min, as were SDS-extracted samples. Samples of 75 µl of the acid extracts and 0.1 ml of the SDS-extracts were used for electrophoresis. At the end of the runs, gels were prepared for fluorography by the method of Bonner and Laskey (16). After exposure of the gels to RP-Royal X-OMAT (Kodak) film at -70°, the films were developed and scanned with an E-C slab gel scanning densitometer with a 620 nm wavelength filter (E-C Apparatus Corp., St. Petersburg, Florida). Peak areas were measured by weighing tracings on a precision balance.

RESULTS

Infected or mock infected confluent BSC-1 cells were labeled for one hour with either [^{3}H]lysine or [^{3}H]thymidine at various times after infection. After the pulse period cells labeled with thymidine were treated by the method of Hirt whereby 95-99% of the host DNA is found in the pellet whereas 90% or more of the viral DNA remains in the supernatant fraction (12, 13).

In mock infected cells very few counts are incorporated into the Hirt supernatant fluid at any time tested (Table 1).

Incorporation of labeled thymidine into the cellular DNA found in the Hirt pellet is significantly greater 21 hours after mock infection than at zero time. This can be attributed to stimulation of cellular DNA synthesis by addition of fresh serum at the time of mock infection (10). (In these experiments fresh medium was used in order to minimize variations in the nutrients and metabolites available to the different cultures, although stimulation of cellular DNA synthesis can be depressed by employing depleted medium). The rate of incorporation of label into cellular DNA appears to decrease steadily at times later than 21 hours after mock infection. It should be pointed out that the incorporation of [^{3}H]thymidine into cellular DNA (Hirt pellet) is not precisely reproducible from one sample to another because of difficulties in washing the viscous pellets, and because of variations in the number of cells on each plate. Variations of up to 25% in parallel cultures have been found in our laboratory (12).

Table 1

Rates of Viral and Cellular DNA Synthesis at Different Times After SV40 Infection

Time after Infection (hrs)	SV40 Infection	c.p.m. in Hirt Supernatant ($\times 10^{-3}$)	c.p.m. in Hirt Pellet ($\times 10^{-3}$)	Counts in Supernatant as % of total
0	-	6	180	3
21	-	5	703	0.7
	+	208	321	39
34	-	11	478	2
	+	827	914	48
48	-	12	326	4
	+	855	630	58
72	-	8	280	3
	+	597	189	76

Infected (40 PFU/cell) or mock infected BSC-1 cellsd were labelled for 1 hour with ^{3}H-thymidine (10 µCi/ml), starting at the indicated times after infection. Extraction of DNA and separation into two fractions (supernatant-containing viral DNA, and pellet containing cellular DNA) and treatment for counting are described in "Methods". Each sample represents one 100 mm culture plate (about 3 x 10^{6} cells).

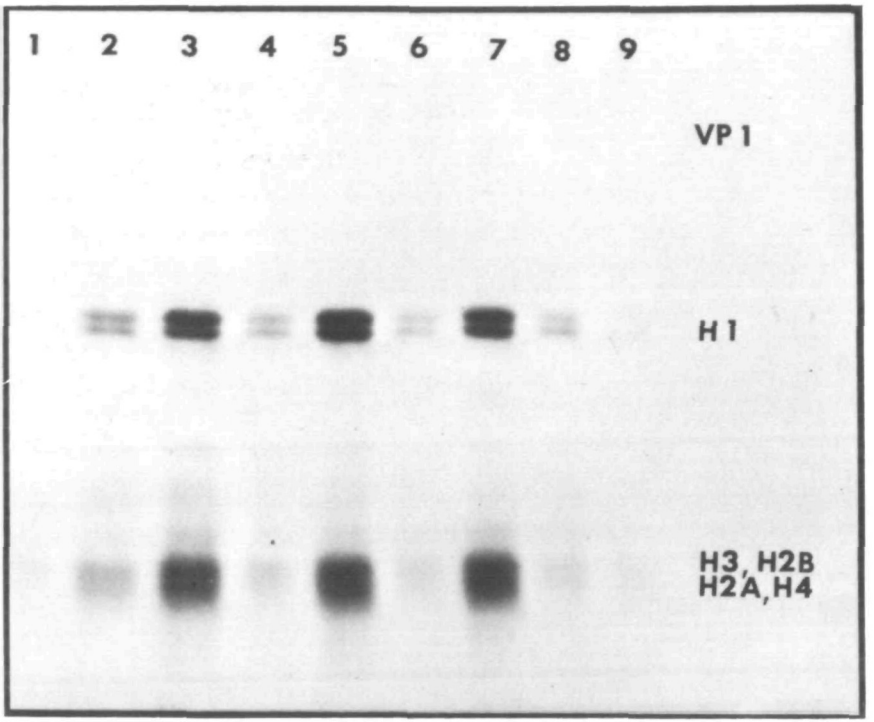
In the cultures which are infected with SV40 significant amounts of [^{3}H]thymidine are incorporated into both the supernatant and pellet fractions. The rate of incorporation of thymidine into cellular DNA is significantly greater 21 hours after infection than at zero time, although it seems to be less than in the equivalent mock infected culture. Also, in infected cells the rate does not decrease after 21 hours. At both 34 and 48 hours after infection the rates of incorporation of [^{3}H]thymidine into cellular DNA in infected cells are about twice those found for equivalent mock infected cells, indicating that SV40 infection is stimulating host DNA synthesis to some extent. This is in accordance with previous observations in our laboratory (12) and with what has been reported by Liberti et al (11). Ritzi and Levine found no stimulation of cellular DNA synthesis after infection of BSC-1 cells with SV40 when experiments were carried out with depleted medium (9). In contrast, cellular DNA synthesis is stimulated up to ten fold by SV40 infection in other cell lines (9).

In the early period of infection, the rate of viral DNA synthesis appears to follow a time course similar to that observed for cellular DNA synthesis. There is significant incorporation of [^{3}H]thymidine into viral DNA at 21 hours post infection and the rate is substantially increased by 34 hours after infection. However, at later times, the high rate of viral DNA synthesis continues while cellular DNA synthesis declines to a level similar to that seen at zero time. By 72 hours after infection viral DNA synthesis is still at a relatively high rate and comprises the bulk of total DNA synthesis occuring in the culture.

The rates of histone synthesis were measured at the same times after infection as the rates of DNA synthesis. Parallel cultures which had been labelled with [^{3}H]lysine were extracted with acid, and the soluble proteins were electrophoresed on an SDS-polyacrylamide slab gel. The incorporation of [^{3}H]lysine was monitored by fluorography (Figure 1). It is difficult to quantitate samples taken at different times after infection because of possible variations in amino acid pools (see discussion). However, general trends can be noted. At zero time, very little [^{3}H]lysine was incorporated into the histones. Since these cells were confluent and had been in the same medium several days, this was to expected. The rate of histone synthesis in the mock infected cells increased by 21 hours and then appeared to decline somewhat at 34 hours, remaining fairly constant between 48 and 72 hours. These changes in the rates of histone synthesis parallel the changes in the rate of incorporation of [^{3}H]thymidine into cellular DNA observed in the mock infected cells (Table 1). In infected cells, the

Figure 1

Fluorogram of [^{3}H]lysine labelled acid soluble proteins extracted at different times after SV40 infection.



BSC-1 cells were infected (40 PFU/cell) or mock infected, labelled for 1 hour with [^{3}H]lysine at various times after infection, extracted with acid and the acid extracted proteins were electrophoresed on a 4%/15% SDS-polyacrylamide gel as described in Methods. The gel was prepared for fluorography as described by Bonner and Laskey (16) and exposed to X-ray film for 100 hours. The positions of markers of SV40 VP1 and calf thymus histones H1, H3, H2B, H2A and H4 are shown. 1, zero time; 2, 4, 6 and 8 - mock infected cells 21, 34, 48, and 72 hours after infection respectively; 3, 5, 7 and 9 - corresponding infected cells. Each sample represents about 5.5 x 10^5 cells.

rate of synthesis of all classes of histones increased greatly by 21 hours after infection in contrast to the corresponding mock infected cells, even though at this time total incorporation of [^{3}H]thymidine into DNA (both Hirt supernatant and pellet fractions) was greater in the mock infected cells than in infected cells (Table 1). After 34 hours, the rate of histone synthesis in infected cells increased relative to 21 hours after infection. By 48 hours after infection, the rate of histone synthesis appeared to decrease and by 72 hours after infection it was less than in the equivalent

mock infected culture even though SV40 DNA synthesis was still relatively high and total DNA synthesis was much greater than in the mock infected culture (Table 1). A possible explanation for the small amount of labeled protein extracted from the cells at 72 hours after infection is that cell death is occuring rapidly and newly synthesized material is being liberated into the medium, resulting in an apparent reduction of labeled protein in the cells. However, visual examination showed no extensive detachment of the monolayer and no significant amount of acid precipitable counts could be found in the medium at any of the times monitored.

After fluorography, the gel shown in Fig. 1 was stained with coomassie blue. Inspection indicated that infected cultures contained far more histone than mock infected cultures, showing that net synthesis of histones had occurred as well as an increased rate of synthesis. The 72 hour infected sample also showed a high histone content, indicating that the low level of incorporation of [^{3}H]lysine into histones at this time is not a reflection of massive degradation of the cellular histones. The possibility that at this time newly synthesized histone is rapidly degraded while preexisting histone is unaffected cannot be excluded.

In growing uninfected cells, histone synthesis is tightly coupled to DNA synthesis (6, 7). If DNA synthesis is interrupted, histone synthesis rapidly stops (7). There are some indications that the converse may also be true (17, 18). In the previous experiment, however, a large increase in histone synthesis was observed 21 hours after infection although SV40 DNA synthesis was far from maximal and total DNA synthesis was less than in mock infected cells. The rate of histone synthesis was maximal at 34 hours after infection and decreased somewhat at 48 hours after infection. Because of the decline in the rate of cellular DNA synthesis, total DNA synthesis in the infected cells was less at 48 hours than at 34 hours after infection even though the rate of SV40 synthesis remained about the same. The decrease in the rate of histone synthesis may reflect the decline of total DNA synthesis. However, at 72 hours after infection histone synthesis had decreased to a level similar to that seen at zero time even though SV40 DNA synthesis is remained at a high rate. Thus the results both early and late after infection suggest that rates of histone synthesis in response to SV40 infection do not have a tight temporal relationship to rates of SV40 DNA synthesis.

To further examine the relationship between the synthesis of histone and of SV40 DNA, we studied at the rates of DNA and histone synthesis earlier than 20 hours and later than 48 hours after infection (Fig. 2 and Table 2).

Table 2

Rates of Viral and Cellular DNA Synthesis Early and Late in SV40 Infection

Time after infection (hrs)	SV40 infection	c.p.m. in Hirt Supernatant (x 10^{-3})	c.p.m. in Hirt Pellet (x 10^{-3})	Counts in Supernatant as % of total
11	+	0.6	69.5	0.9
14	+	0.9	96	0.9
17	+	2	121	2.0
19	+	6	170	3.6
49	+	96	145	40
52	+	162	150	52
55	+	110	134	45
57	+	142	175	45

Experimental details were as in legend to Table 1, except that 1 μCi/ml of [^{3}H]thymidine was used.

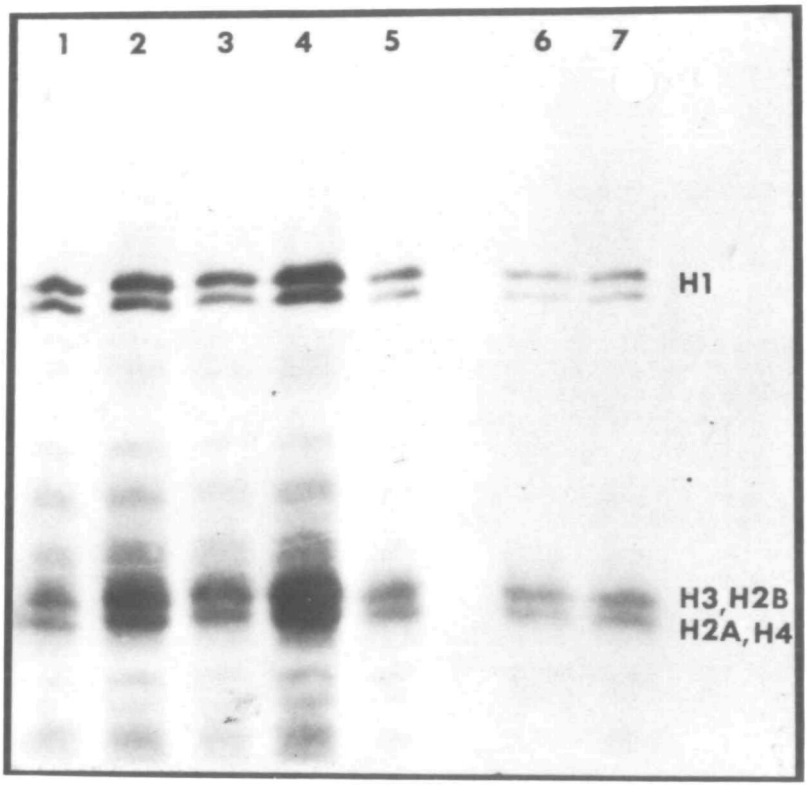
From 11 to 19 hours after infection, the rates of cellular DNA synthesis increased. SV40 DNA synthesis, as measured by [^{3}H]thymidine in the Hirt supernatant fraction, could not be detected earlier than 17 hours after infection and the rate was very low even at 19 hours after infection. Nevertheless, as shown in figure 2, histone synthesis was already considerable by 11 hours after infection and the rate increased substantially 19 hours after infection.

The situation is different late in the infectious cycle. From 49 to 57 hours after infection the rates of cellular DNA synthesis were as high as those observed at 19 hours after infection. The rates of viral DNA synthesis at these times were also high. However, the rates of histone synthesis were much lower than the rates observed early in infection.

Therefore late in infection there is an obvious dissociation between the rates of histone synthesis and the rates of DNA synthesis as suggested by the experiment described in Table 1 and Figure 1. Interpretation of the situation early in infection is more difficult since the increased rate of histone synthesis occurs during a period when host DNA synthesis is also

Figure 2

Fluorogram of [^{3}H]lysine-labeled acid soluble proteins extracted early and late in SV40 infection



Methods as in legend to Fig. 1. Exposure of gel to X-ray film was for 1 week. All samples are from infected cells. Sample 1-7: 11, 14, 17, 19, 49, 55 and 57 hours after SV40 infection. All samples represent about 4×10^{5} cells.

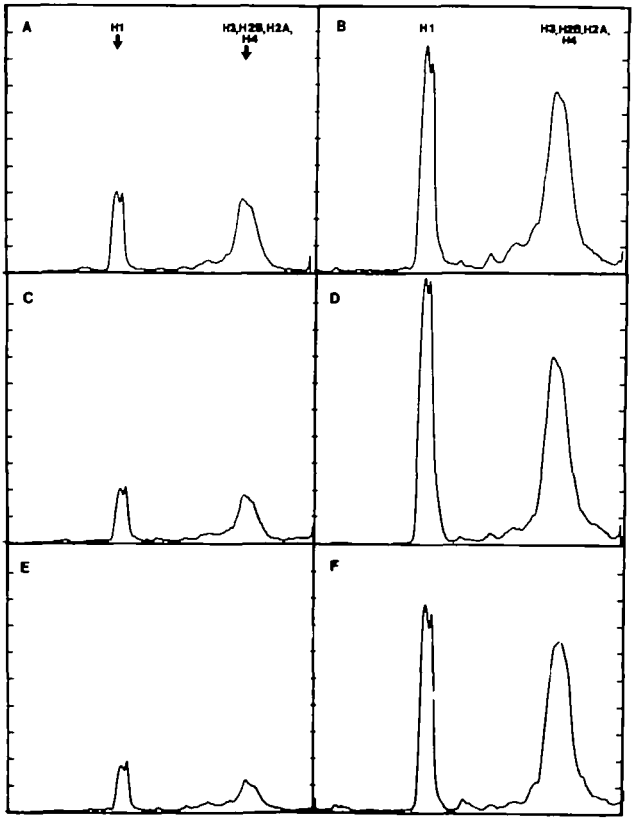
increasing. However, the rates of host DNA synthesis between 11 and 19 hours after infection are less than or equal to those observed between 49 and 57 hours post infection (Table 2). Assuming that the temporal relationship between cellular DNA synthesis and histone synthesis remains true and that similar rates of cellular DNA synthesis elicit similar rates of histone synthesis, then the contribution of host DNA synthesis to the rates of histone synthesis observed between 11 and 19 hours after infection cannot be more than the contribution of host DNA synthesis to those rates between 49 and 57 hours after infection (and may be less if part of the histone

synthesis late in infection is related to SV40 DNA synthesis). At all times monitored between 11 and 19 hours after infection the rates of histone synthesis are greater than those observed late in infection, suggesting that a substantial part of the early increase in the rates of histone synthesis, even at times when SV40 DNA synthesis is undetectable, may be the result of SV40 infection itself rather than the accompanying stimulation of cellular DNA synthesis.

The graphs in Fig. 3, which are densitometer scans of the fluorograms shown in Fig. 1, suggested that as infection proceeded the H1 peak increased relative to that of the other histones, reaching a maximum 34 hours after

Figure 3

Densitometer scans of fluorogram of acid soluble [^{3}H]lysine-labeled proteins extracted from mock infected or SV40 infected cells.



Densitometer scans of the fluorogram shown in Fig 1. A, C, E: from mock infected cells labeled 21, 34 and 48 hours after infection; B, D, F: from corresponding infected cells. All scans are to same scale.

infection (see Table 3). In mock infected cells the amount of [^{3}H]lysine incorporated into H1 appeared to remained constant compared to incorporation into the other histones.

However, Olins et al. (19) have reported that acid extractions of histones are often incomplete, and may therefore influence the observed ratios of the different histones to one another. Furthermore, it is possible that although the labeling time was too short to allow release of labeled virus into the medium, viral structures which are resistant to acid extraction may be formed within the cell. It should be noted that the capsid proteins of SV40 have pI's which are slightly acidic (20) and our gels of acid extracted protein show little or no labeled protein migrating at the positions of the capsid proteins even at 34 or 48 hours after infection when these proteins are being synthesized in large amounts. Since the four smaller histones are incorporated into the virion, but H1 is not, any sequestering of histones into acid resistant viral structures would lead to an apparent increase in the ratio of H1 to the other histones.

To clarify these questions, infected and mock infected cells were extracted with SDS-urea-2-mercaptoethanol and the extracts electrophoresed. The gel was monitored by fluorography and scanning (Fig. 4).
The histones are among the most highly labeled proteins in the extracts and can be readily identified. At 19.5 hours after infection little or no

Table 3

Relative of Labeled H1 and Other Histones in Acid and SDS Extracts of Infected Cells

Time after Infection (hrs)	SV40 Infection	Intensity of H1 Peak / Intensity of H3+H2B+H2A Peaks	
		Acid Extraction	SDS EXtraction
21	+	0.6	
34	+	0.9	
48	+	0.6	
19.5	-		0.9
	+		0.8
37.8	-		0.7
	+		0.5
45.3	-		0.7
	+		0.6

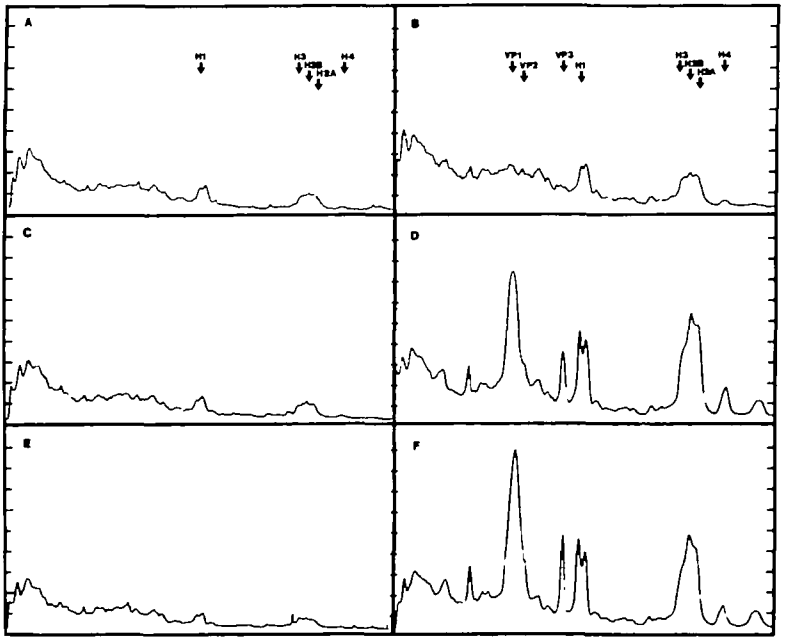
The peak areas of H1 and H3+H2B+H2A were determined from the densitometer scans shown in fig. 3 and 4.

radioactivity is detected in viral capsid proteins, but these proteins are major components 38 hours after infection and their rate of synthesis is even greater 45 hours after infection. Histone synthesis also increased from 19 to 38 hours after infection and began to decrease by 45 hours post infection. The difference in rates of histone synthesis between mock infected and infected cells early in infection (19.5 hours) is less than in previous experiments. However, a lower multiplicity of infection was used, and viral DNA synthesis is a function of multiplicity up to about 50 PFU/cell (27, 28).

In the extracts obtained by SDS treatment no increase in HI relative

Figure 4

Densitometer scans of fluorogram of [^{3}H]lysine-labeled SDS-extracted proteins from mock infected or SV40 infected cells.



BSC-1 cells were infected (10 PFU/cell) or mock infected, labeled for 1 hour with [^{3}H]lysine at various times after infection, extracted with SDS-2-mercaptoethanol and electrophoresed on a 3%/18% SDS-polyacrylamide gel as as described in Methods. The gel was fluorographed (16) with a 3 day exposure, and the X-ray film was scanned. A, C, E.: mock infected cells labeled 19.5, 37.8 and 45.3 hours after infection; B, D, F.: corresponding infected cells. All scans are to the same scale but are not directly comparable to Fig. 3.

to the other histones is apparent: if anything, relative H1 content decreases (Table 3). There is some variation in the ratios, but more protein is extracted with SDS than with acid increasing background levels and making it more difficult to calculate peak intensities accurately.

H1 is reported to be present in only about half the molar amount of the other histones in most tissues (21, 22, 23), according to determinations made on acid extracts. However, Olins et al. (19) reported that when chick erythrocytes are extracted with SDS-2 mercaptoethanol, the lysine rich histones (H1 plus H5) are detected in amounts equimolar to the other histones. In calf thymus, H1 has 61 lysine residues per molecule while H3, H2A and H2B have only 47 combined (24). Assuming that the distribution of lysine residues is similar in histones from BSC-1 cells then if all histones are synthesized in equimolar amount, the incorporation of labeled lysine into H1 is expected to be 1.3 times that incorporated into H3, H2A and H2B together. Our results (Table 3) suggest rather that in both mock infected and infected cells, H1 is synthesized in about half the amount of the other histones.

DISCUSSION

We have shown that lytic infection of resting BSC-1 cells with SV40 stimulates histone synthesis. In mock infected cells, what histone synthesis there is appears to follow closely the incorporation of label into cellular DNA, as is generally the case in growing non-infected systems (6, 7). In infected cells, however, histone synthesis in response to SV40 infection appears to begin and to decline several hours before similar events can be detected in the rate of SV40 DNA synthesis.

Correlation of rates of protein or DNA synthesis at different times, especially in a lytic viral infection, is complicated by the possibility of fluctuations in the nucleotide or amino acid pools and the problem of equilibration of added labeled precursors with pre-existing pools. We have not studied these problems extensively. However, 37 hours after infection, incorporation of thymidine into SV40 DNA is linear for up to one hour, with no lag detected even at the earliest time point, indicating that equilibration of the [^{3}H]thymidine with pre-existing pools occurs quickly (unpublished observations). Furthermore, Liberti et al. (11) have shown that in CV-1 monkey kidney cells, incorporation of labeled precursor into viral DNA is accompanied by a proportional increase in the viral DNA mass until about 48 hours after infection and in BSC-1 cells, the time course of infection is longer than in CV1-cells (9, 28). Thus, significant variations in the

nucleotide pool may only occur late in infection, when virions are being released. The decline of incorporation of [^{3}H]lysine into histones later than 35 hours after infection is probably not explained by an increased intracellular pool of lysine since although incorporation of the label into histone declines between 38 and 45 hours post infection, incorporation into the viral proteins increases (Fig. 4). Also, the background incorporation into proteins other than the viral proteins and histones (Fig. 4) remains fairly constant from 19 to 45 hours after infection. We think therefore that possible fluctuations in intracellular pools have not significantly affected our results.

Histone synthesis is certainly depressed at times later than 48 hours after infection, when SV40 DNA synthesis continues at a high rate. Early in infection, the situation is complicated by the rise in cellular DNA synthesis up to 20 hours after infection. However, the rate of incorporation of [^{3}H]thymidine into cellular DNA at 49 to 57 hours after infection is greater than or equal to the rate of incorporation early in infection (Table 2). The rate of histone synthesis related to host DNA synthesis early in infection should therefore be less than or similar to that elicited by host DNA synthesis late in infection. However, the rates of histone synthesis observed from 11-19 hours after infection are very much higher than the rates obtained from 49-57 hours after infection (Fig. 2). The rate of histone synthesis early in infection does not seem to be commensurate with the rate of cellular DNA synthesis, suggesting that histone synthesis is stimulated by SV40 infection. However, SV40 DNA synthesis cannot be detected before 17 hours after infection (Table 2), and then it is at a very low rate. Therefore the increase in histone synthesis in response to SV40 infection is observed several hours before the onset of detectable SV40 DNA replication and also decreases to baseline levels at a time when SV40 DNA replication is still substantial.

There are several possible reasons for such a dissociation of histone synthesis from DNA synthesis. Late in infection the synthesis of large amounts of viral capsid proteins may require most of the protein synthetic apparatus of the cell, resulting in a decrease of histone synthesis before viral DNA synthesis is completed. The early start in histone synthesis may result in larger than normal histone pools which can be used for the SV40 DNA synthesized after histone synthesis is almost completely shut down late in infection. Another possibility is that infecting viral DNA, which lacks H1 (since H1 is not detected in virions), needs H1 before it can begin

replication. If the genes for all five histones genes are expressed coordinately, then synthesis of all histones may be turned on even if only H1 is needed. Later in infection, as the viral DNA and four smaller histones are encapsidated, H1 may accumulate in the cell and turn off histone synthesis. That histones may regulate their own synthesis has been proposed previously (25).

Synthesis of all five classes of histones are stimulated to about the same extent by SV40 infection, although H1 is not detected in the completed virion. Varshavsky et al showed that H1 can be found in association with the SV40 minichromosome within the infected cell (5). We are continuing studies on the role of H1 during SV40 lytic infection and on whether the H1 synthesized in response to SV40 infection represents any particular one of the H1 subfractions (26). One slight indication that this may be so is the difference in relative proportions of the components of the H1 doublet seen with mock infected and infected cells (Fig. 3 and 4).

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