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Human Cytomegalovirus Stimulates Host Cell RNA Synthesis

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Human cytomegalovirus infection of human fibroblast cells (WI-38) induced cellular RNA synthesis. The RNA synthesis in infected cultures preceded the synthesis of viral DNA and progeny virus by approximately 24 h. RNA species synthesized in infected cells included ribosomal 28*S* and 18*S*, and 4*S* transfer RNA; all were markedly increased in comparison to uninfected cells. This induction of host cell RNA synthesis was dependent upon a protein(s) that was synthesized during the early stages of infection.

The usual effect of infection with herpes group viruses is a reduction of the synthesis of cellular RNA and a decrease of polysomes. Both herpes simplex virus (1, 10, 13, 28) and pseudorabies virus (15, 26) infection lead to a gradual decrease in the rate of cellular RNA synthesis beginning within hours postinfection (PI) and the onset of progeny virus DNA synthesis by 3 to 4 h PI (15).

The replication kinetics of human cytomegalovirus (CMV), another herpes group virus, have been studied in productively infected WI-38 human fibroblasts (11). In these cells there is rounding of infected cells within 6 to 12 h, but viral DNA is not synthesized until 48 h PI, soon followed by the release of infectious virus and ultimately cell death (11, 14). Taking advantage of this relatively long latent period for CMV replication, we have studied the effect of CMV infection on host cell macromolecular synthesis during the period prior to release of the infectious virus. In the present paper, we describe the effect of CMV infection on RNA synthesis in WI-38 host cells. The results of these experiments show the induction of host cell RNA synthesis including ribosomal 28*S* and 18*S*, and 4*S* RNA. This induction was mediated by a protein(s) synthesized in the early stage of infection.

MATERIALS AND METHODS

Cells and viruses. The human CMV strain (Towne) and the host cell WI-38 cells have been previously described (11). WI-38 cells were seeded in tissue culture dishes (Falcon) with Eagle minimal essential medium supplemented with 10% fetal bovine serum and antibiotics. The cells were infected 3 days after confluence with 10 PFU of CMV per cell which was permitted to absorb to cells for 1 h at 37 C, after which cultures were refed with fresh medium containing 2% fetal bovine serum. Under these conditions, all the cells were infected (11).

Radioactive labeling of cells. To label RNA, [^{3}H]uridine (40.4 μCi/mM; New England Nuclear) was added to cultures pulse-labeled for 2 h. The labeling conditions for individual experiments are given in the legends to figures and tables. After labeling, the cultures were washed twice with ice cold phosphate-buffered saline (PBS) (pH 7.2), then lysed with 1% sodium dodecyl sulfate (SDS) in PBS. The lysate was brought to a 5% trichloroacetic acid concentration by adding cold 10% trichloroacetic acid, and acid-insoluble materials were collected on nitrocellulose membranes, washed with 5% trichloroacetic acid and dried, and the radioactivity was measured.

Separation of cytoplasmic and nuclear RNA. At the termination of pulse labeling, cells were collected in PBS by scraping and pelleted by centrifugation. The cells were washed twice with ice cold PBS and resuspended in 0.02 M Tris-hydrochloride (pH 7.4) and 0.001 M EDTA (TE buffer) at concentrations of 2 to 4 $\times$ 10^6/ml. To lyse the swollen cells, Nonidet P-40 was added to a final concentration of 0.5%. Nuclei were separated by centrifugation and washed twice with TE buffer containing 0.1 M NaCl (TES). The Nonidet P-40 extracts and nuclear suspensions were added to 5% SDS solutions (final concentration of 1% SDS) and then mixed with an equal volume of 80% phenol saturated with TES-buffer. The RNA was extracted three times with phenol, precipitated with 2 volumes of cold ethanol, and kept overnight at 20 C.

Sucrose gradient centrifugation of cytoplasmic and nuclear RNA. To separate and characterize RNA species being synthesized, CMV-infected cells were harvested at 32 and 62 h PI and the RNA was extracted from the nuclear and cytoplasmic fractions. The cells were pulse labeled for 2 h with [^{3}H]uridine (50 μCi/ml of culture medium) at 30 and 60 h PI. The RNA was extracted from the nuclei of infected cells and from the cytoplasmic fraction by the procedures described above and analyzed by velocity sedimentation in 5 to 20% (wt/wt) linear sucrose gradients in TES-buffer containing 0.1% SDS. Centrifugation was at 48,000 rpm at 18 C for 3 h for RNA from cytoplasm, 2.5 h from nuclei, in the SW50 rotor. The 5-ml gradients were fractionated into 0.2-ml portions and acid-insoluble radioactivity of a 10-μliter portion of

each fraction was measured by precipitation in 2 ml of ice cold 5% trichloroacetic acid in the presence of 100 μg of carrier yeast RNA.

Preparation of viral DNA and DNA-RNA hybridization. Purified CMV DNA (for annealing with RNA from CMV-infected cells) was obtained from 200 ml of culture medium containing 10^7 PFU of infectious CMV per ml. Virus-containing medium was centrifuged at 20,000 rpm for 90 min in SW25.1 rotor, and the pellet was suspended in 2 ml of TES and layered on a linear gradient made of 15 to 60% sucrose solution in TES. Centrifugation was 24,000 rpm at 4 C for 90 min in a Spinco SW25.1 rotor as described previously (11). Fractions containing CMV were pooled, diluted to 28 ml with TES, and resedimented at 20,000 rpm for 90 min. The partially purified virus pellet was suspended in 5 ml of TSM (0.02 M, Tris-hydrochloride; 0.01 M, MgCl; 0.1 M NaCl) and treated with DNase (50 μg/ml, Worthington Biochemical Corp.) for 1 h at 37 C, then repelleted by centrifugation at 48,000 rpm for 60 min in a SW50 rotor at 4 C, and suspended in 2 ml of TES to which was added 0.5 ml of 5% SDS. CMV DNA was extracted three times with equal volume of 90% phenol and precipitated by alcohol. DNA was dissolved in 1 ml of TES and treated first by RNase (heat treated, 30 μg/ml, Worthington Biochemical Corp.) for 1 h at 37 C, then pronase (heat treated, 100 g/ml, Calbiochem) for 2 h at 37 C. CMV DNA was then extracted twice with phenol and precipitated by alcohol.

The DNA to be used for RNA hybridization was further purified by isopycnic centrifugation in CsCl gradient. Viral DNA obtained after centrifugation had a buoyant density of 1.718 g/cm. Viral DNA fractions (1.715- to 1.720-g/cm^3 fractions) were pooled and dialysed against 0.1 × SSC (0.15 M NaCl plus 0.015 M sodium citrate) at 4 C, diluted to 20 μg/ml, and stored at −20 C. A modification of the procedure of Gillespie and Spiegelman (12) described by Wagner and Roizman (30) was employed for RNA-DNA hybridization. Briefly, CMV DNA in 0.1 × SSC was denatured by heating at a concentration of 1 μg of CMV-DNA per ml for 10 min at 100 C, followed by rapid cooling in an ice bath. The solution was adjusted to a final concentration of 1 μg of CMV-DNA per ml in 4 × SSC, and 10 ml of the solution were filtered through a 25-mm nitrocellulose-filter (Schleicher-Schuell) by gravity. The filter disks were dried, then used for incubation with [^{3}H]uridine-labeled RNA fractions from sucrose velocity sedimentation in 1 ml of 4 × SSC at 67 C for 20 h. The washing and RNase hydrolysis of the filter disks were carried out according to Wagner and Roizman (30).

Preparation of cytoplasmic extract and analysis of polysomes. To detect CMV-specific polysome formation, 4 × 10^6 infected cells were labeled for 2 h at 30 and 60 h PI with [^{3}H]uridine (50 μCi/ml). The labeled cells were collected by scraping in 2 ml of cold PBS and pelleted by centrifugation at 2,000 rpm in the International PR-2 centrifuge, and washed twice with cold PBS. To lyse cells, Nonidet P-40 was added to a final concentration of 0.5% during a brief, vigorous mixing. Nuclei were removed by low speed centrifugation. The extract was then treated with 1% sodium deoxycholate for 5 min at 4 C and layered on top of 30 ml of 15 to 30% linear sucrose gradient and centrifuged for 2 h at 24,500 rpm in a SW25.1 rotor. After centrifugation, a 1-ml fraction was collected from the bottom of the tubes for the measurement of radioactivity.

Measurement of acid-soluble and acid-insoluble radioactivity in RNA. Cells (5 × 10^5) were labeled for 2 h with 1 μCi of [^{3}H]uridine per ml at different times after CMV infection. The cultures were rinsed three times with cold PBS and suspended in 2 ml of ice cold 5% trichloroacetic acid by scraping with a rubber policeman. The precipitate was collected by low speed centrifugation, suspended in 2 ml of cold, fresh 5% trichloroacetic acid, and recentrifuged. The supernatants from two successive centrifugations were combined and regarded as the acid-soluble fraction. A small portion (0.1 ml) of the fraction was spread on a 25-mm glassfiber filter disk, dried under infrared lamp which represented acid-soluble radioactivity.

RESULTS

Synthesis. Rather than the expected inhibition, host cell RNA synthesis in CMV-infected cells was found to be stimulated. A typical experiment is illustrated in Fig. 1, in which it can be seen that the rate of RNA synthesis remained unchanged in infected cells during early hours of CMV infection. At approximately

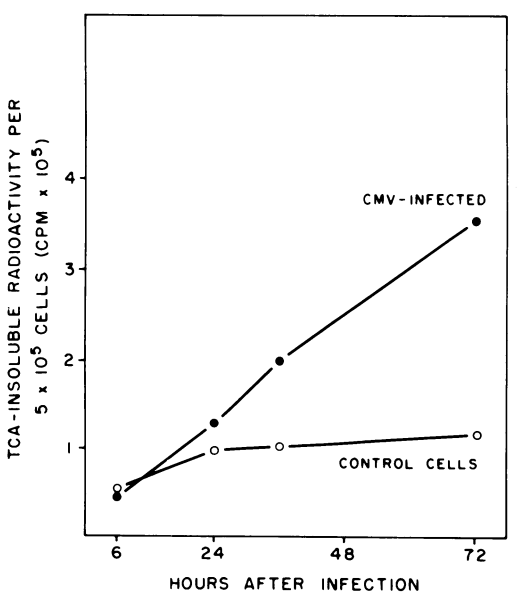

FIG. 1. *WI-38 cells were labeled with 10 μCi of [^{3}H]uridine per ml for 60 min at various times after CMV infection. The trichloroacetic acid-insoluble radioactivity was measured by the procedures described in the text. Symbols: ●, CMV-infected cells; ○, mock-infected cells.*

24 h PI, however, the rate of RNA synthesis in the infected culture began to slightly exceed that of the control culture (14). This rate of host cell RNA synthesis in infected cells continued to increase until the onset of viral DNA synthesis that occurs at 48 to 72 h PI (11). The accumulation of radioactive counts was observed in both the nuclear and cytoplasmic fractions (Fig. 2a and b).

Effect of rifampin on CMV-induced RNA synthesis. Rifampin derivatives have been reported not to inhibit early mRNA, late mRNA, or viral DNA formation of vaccinia virus (7, 18). However, some reports have indicated a partial inhibition of RNA synthesis (5, 20). We have observed that there was practically no incorporation of labeled precursor into viral DNA and no production of progeny virus when rifampin was present in the culture medium (100 μg/ml) (9). However, if rifampin was removed after a 48-h treatment, the replication of CMV was restored. This result suggested that rifampin-treated cells had not lost the ability to replicate CMV. We tested the effect of rifampin on the CMV-induced host cell RNA synthesis and the results are shown in Table 1. The stimulation of [^{3}H]uridine incorporation into host RNA was apparent in CMV-infected cells, with incorporation approximately 5.5-fold that of controls. The extra stimulation was reduced to control values by rifampin.

Location and size of newly synthesized RNA species in CMV-infected cells. To identify the newly synthesized RNA, cultures infected with CMV both in the presence and absence of rifampin, and uninfected cultures were exposed to [^{3}H]uridine for 2 h at various times during the infectious cycle. The RNA was extracted from cytoplasmic and nuclear fractions of cells and analyzed by sucrose gradient centrifugation.

A comparison of the rate of [^{3}H]uridine incorporation into different subcellular fractions is illustrated in Fig. 2. The stimulation of the incorporation was clear at 36 h PI in both cytoplasmic and nuclear fractions, and the sensitivity to rifampin was essentially the same in both.

RNAs extracted from both cytoplasmic and nuclear fractions were analyzed by sucrose density gradient centrifugation. The profile of RNA patterns is shown in Fig. 3a and b. RNA extracted from nuclear fraction both at 30 and 60 h (Fig. 3a) contained significantly more 45*S* RNA in CMV-infected cells than in uninfected cells. That cellular RNA synthesis was induced in CMV-infected cells was also supported by the cytoplasmic RNA pattern of infected cells (Fig. 3b): by 30 h PI, 28*S*, 18*S*, and 4*S* RNA were increased markedly over control cells; at 60 h PI, there was a 10-fold increase of each RNA peak in infected cells.

Hybridization of RNA from infected cells with CMV DNA. Fractions from sucrose gradient sedimentation of cytoplasmic RNA were pooled and collected into five-fraction pools. The approximate *S* value of the main component of each pooled fraction is shown in the second column of Table 2. RNAs were hybridized to CMV DNA which was immobilized to nitrocellulose filter disks, and RNase-resistant radioactivities on the disks were assayed after

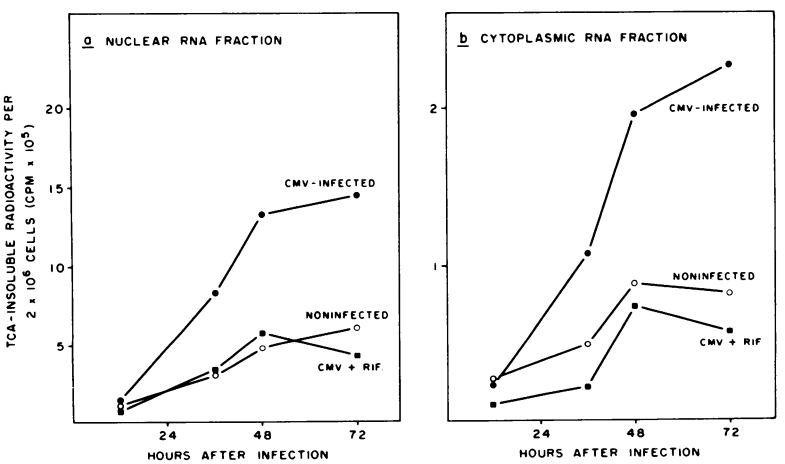

FIG. 2. *Comparison of the rate of labeling of nuclear and cytoplasmic RNA between CMV-infected WI-38 cells in the presence (■) and absence (●) of 100 μg of rifampin and of mock-infected cultures per ml at different times PI. The nuclear and cytoplasmic RNA were extracted by phenol-SDS and precipitated as described in the text.*

TABLE 1. *Incorporation of [^{3}H]uridine into CMV-infected and mock-infected WI-38 cells[a] in the presence of rifampin*

Infection	Rifampin (100 μg/ml)	[^{3}H]uridine-incorporated counts/min per culture (2 × 10^6 cells)
Mock	−	32,210
Mock	+	10,270
CMV	−	178,330
CMV	+	45,125

[a] Cells were labeled for 2 h with 50 μCi of [^{3}H]uridine per ml at 36 h after the CMV infection. Cytoplasmic RNA was extracted from Nonidet P-40-treated cell lysates by phenol-SDS method.

20 h of hybridization. At 30 h PI, there was virtually no hybridization. At 60 h PI, the RNA fraction that appeared in the 12 to 16S region showed the most significant hybrid formation to CMV DNA. Although the technique is not sensitive enough to allow firm conclusions, it appeared that relatively more RNA in other fractions was host cell RNA species.

Polysome formation in CMV-infected cells. To see if the cellular polysome pattern also changed concomitantly with the appearance of the newly synthesized ribosomal RNAs, cytoplasmic extracts were made by treating cells with 0.5% Nonidet P-40 and 1% sodium deoxycholate. [^{3}H]uridine-labeled extracts were then analyzed by sucrose gradient sedimentation. Cytoplasmic extract made at 30 h PI contained five times more polysomes and, at 60 h almost ten times that of uninfected cell extract (Fig. 4). This stimulation of polysome formation implies synthesis of considerable amounts of new proteins in CMV-infected cells.

Uptake of [^{3}H]uridine in CMV-infected and uninfected cells. To determine whether increased pool size of [^{3}H]uridine would account for the apparent difference in RNA synthesis in infected and mock-infected cultures, the intracellular distribution of [^{3}H]uridine was compared at different periods of infection (Table 3). Acid-insoluble radioactivity increased significantly in infected cultures 24 h PI, acid-soluble radioactivity did not. There was about a four-fold increase in the rate of RNA synthesis in infected cultures compared to uninfected culture at 48 h, whereas there was only a 50% increase of [^{3}H]uridine uptake. In addition, the inhibitory effect of cycloheximide on protein synthesis was more pronounced on the incorporation into the acid-insoluble fraction than into the acid-soluble fraction. In infected cultures at 48 h PI, acid-insoluble radioactivity had decreased 60% in the presence of cycloheximide, but acid-soluble radioactivity decreased only 15%. These results showed that the induction of cellular RNA synthesis in CMV-infected cells

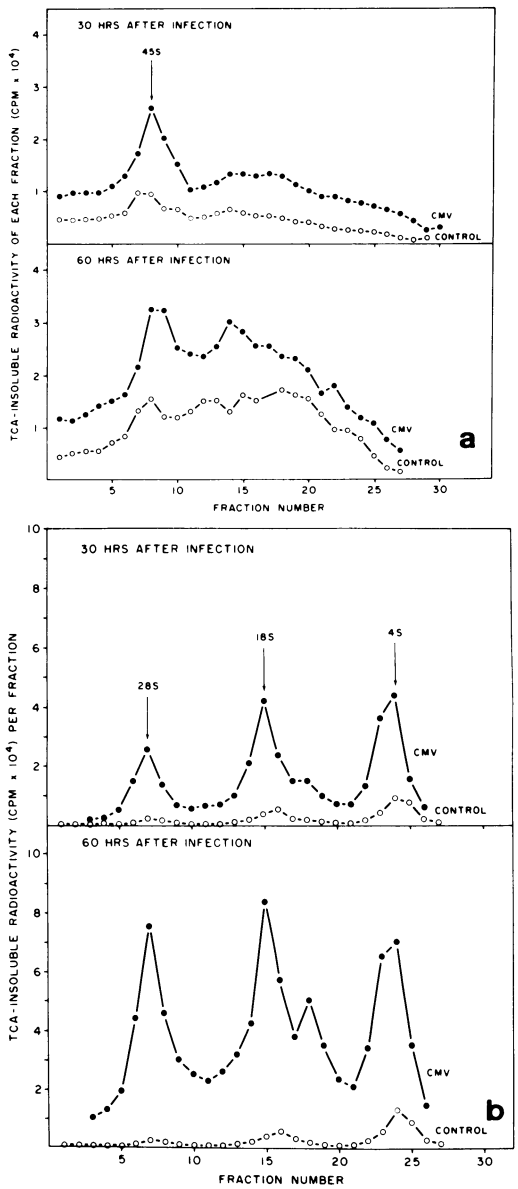

FIG. 3. *Sedimentation distribution of cytoplasmic and nuclear RNA synthesized in WI-38 cells infected with CMV 30 and 60 h PI. The cultures were incubated for 60 min in medium containing 50 μCi of [^{3}H]uridine per ml and nuclear and cytoplasmic RNA were extracted by phenol-SDS. The extracted RNA was precipitated with ethanol and centrifuged in a 5 to 20% (wt/wt) sucrose gradient for 3 h at 4 C and 48,000 rpm in a Spinco SW50 rotor. The RNA was precipitated with trichloroacetic acid and assayed for radioactivity. (a) RNA from nuclear fraction; (b) RNA from cytoplasmic fraction.*

was not due to the increase of intracellular distribution of radioactive precursor, and that the majority of such induced RNA was sensitive to inhibition of protein synthesis.

Early effect of cycloheximide on the induction of cellular RNA synthesis. To provide direct evidence of a relationship between the induction of cellular RNA synthesis and the replication mechanisms of CMV, the effects of cycloheximide on cellular RNA synthesis and on the production of progeny virus were examined. CMV-infected cultures were treated with 2 μg of cycloheximide per ml for 6- or 12-h periods at different times PI. After each treatment, the culture medium containing cycloheximide was removed and replaced with fresh medium. The cultures were then incubated and RNA synthesis and virus titer were assayed at 48 and 72 h PI, respectively (Table 4).

When the infected cells were treated during 0 to 6 h PI, there was an inhibitory effect of cycloheximide on RNA synthesis at 48 h; treatment at later stages was not inhibitory. The most "cycloheximide-sensitive" period was the first 6 h of infection during which the drug inhibited RNA synthesis as well as virus replication. Treatment at later stages of infection with cycloheximide did not much affect RNA synthesis measured at 48 h PI. Indeed cycloheximide-treated uninfected cultures showed increased RNA synthesis compared to untreated controls, although not to the levels observed with infection. Although inhibition of virus production was evident in all drug-treated cultures, the recovery of virus production was rapid when the treatment was applied late in infection.

DISCUSSION

The results presented in this communication show that CMV infection induces the synthesis of cytoplasmic RNAs including ribosomal 28 and 18S RNA and tRNA, and of nuclear RNAs. The onset of induction of cellular RNA synthesis begins approximately 24 h after CMV infection, preceding the synthesis of infectious virions and viral DNA which occur 48 h after infection. However, as we reported previously, there is rounding of infected cells and an early protein is synthesized at 2 to 6 h PI, well before the induction of cellular RNA synthesis (11).

The mechanisms of induction of cellular RNA synthesis in CMV-infected cells are not yet

TABLE 2. *Size distribution of cytoplasmic RNA specified by CMV DNA*

Fraction	Approximate S value	[^{3}H]uridine input (counts/min)	RNase-resistant [^{3}H]uridine[a] (counts/min)
I	28S	73108	136 (0.186)
II	20 to 24S	40988	132 (0.322)
III	18S	79048	228 (0.288)
IV	12 to 16S	66188	512 (0.798)
V	4S	104348	108 (0.09)

[a] Values in parentheses indicate percentage.

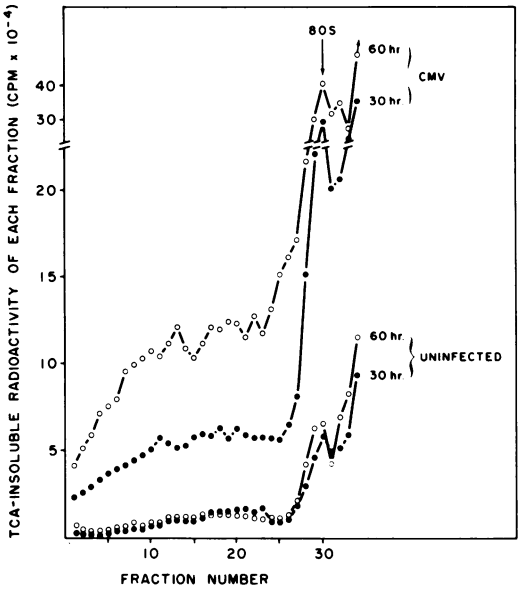

FIG. 4. *Polysome pattern of CMV-infected and mock-infected WI-38 cells at 30 h (●) and 60 h (○) PI. For details, see text.*

TABLE 3. *[^{3}H]uridine pool size in CMV-infected and mock-infected cells in the presence and absence of cycloheximide*[a]

Time and fraction	Cultures			
	CMV-Infected		Mock-Infected	
	Without cycloheximide	With cycloheximide	Without cycloheximide	With cycloheximide
6 h				
TA-soluble	92,200	85,420	92,940	86,440
TA-insoluble	3,005	2,628	2,837	2,486
24 h				
TA-soluble	131,360	102,220	122,400	96,120
TA-insoluble	10,399	4,362	3,556	3,127
48 h				
TA-soluble	236,580	200,060	159,800	126,040
TA-insoluble	16,207	6,830	4,136	2,666

[a] Counts per minute per culture of 5×10^5 WI-38 cells. Cells were labeled for 2 h with 1 μCi of [^{3}H]uridine per ml in the presence or absence of cycloheximide (50 μg/ml). TA, Trichloroacetic acid.

TABLE 4. *Effect of short periods of cycloheximide treatment on cellular RNA synthesis and production of progeny CMV*

Periods of treatment after CMV infection or mock-infection	RNA synthesis[a]				CMV progeny production[b]
	Mock-infected	Mock-infected + cycloheximide[c]	Infected	Infected + cycloheximide	Infected + cycloheximide[c]
0 to 6 h	11,118	13,691	38,789	18,369	1.0×10^1
0 to 12 h	14,647	22,036	39,803	11,621	1.0×10^1
6 to 12 h	13,959	21,225	38,024	35,202	1.5×10^3
12 to 24 h	14,118	21,134	38,819	33,845	2.0×10^3
24 to 36 h	12,780	17,102	38,483	30,667	1.3×10^4
None					(5.7×10^4)

[a] In terms of [^{3}H]uridine incorporated into acid-insoluble materials total counts per 5×10^5 cells at 48 h PI.
[b] PFU per milliliter at 72 h PI.
[c] Cycloheximide at 2 μg/ml.

understood but the synthesis of early protein probably plays an important role in this process. This is suggested from the finding of the "early effect" of cycloheximide on RNA synthesis as well as on virus replication (Table 4). Further, we found that when the induction of cellular RNA synthesis and the synthesis of viral DNA were abolished in the presence of rifampin, the removal of the drug from the infected cells resulted immediately in a restoration of cellular RNA synthesis followed by viral DNA synthesis (T. Furukawa, S. Tanaka, and S. Plotkin, manuscript in preparation).

In fact, host cell RNA synthesis may be necessary for the process of CMV replication. Whether, in turn, the protein that is synthesized at an early stage of infection initiates the process of host cell RNA synthesis that begins at 24 h PI is unknown. The induction of cellular RNA species synthesis, namely host cell ribosomal RNA, seems to be a characteristic feature of human CMV. Although some animal viruses, such as adenovirus (8, 16), SV40 (19), and polyoma (2) have been shown to enhance certain host cell RNA species, the enhancement was not on host cell ribosomal and transfer RNA synthesis. The effect of CMV on cellular RNA is clearly different from that of other herpes viruses (10, 13, 15, 26, 28). Usually, herpesviruses inhibit the synthesis of cellular RNA as well as the formation of polysomes (4, 25) within a few hours PI. The abortive infection of AD-12 has been shown to induce host cell ribosomal RNA synthesis with a time course parallel to that of the induction of cellular DNA synthesis (22). Although there was no significant induction of host cell DNA synthesis under the experimental conditions in this report, such induction has been observed under other conditions (24). The mechanism of host cell DNA synthesis is not yet known in these systems; however, we have observed that the induction of host cell DNA synthesis in guinea pig cells abortively infected with CMV was accompanied by cellular ribosomal RNA synthesis (T. Furukawa, S. Tanaka, and S. Plotkin, manuscript in preparation). These observations seem to indicate that CMV causes significant alteration of the host cells control mechanisms through the activation of host cell genome in both productive and nonproductive systems.

The induction of cellular RNA and DNA synthesis through the activation of the cell genome has been observed in cells treated by biologically active factors or materials such as phytohemagglutin- (21, 23), serum- (28, 29), and hormone- (3, 17, 27) treated culture cells. Although similar gene activation is found in CMV-infected cells, the kinetic characteristics of host cell macromolecular synthesis are quite different from those observed in cells stimulated by activating factors. The induction of RNA synthesis in active factor-treated cells becomes significant within 3 to 6 h after treatment, whereas that of CMV-infected cells begins approximately 24 h PI and continues for at least an additional 48 h.

Correlations between transport or specific precursor and synthesis of macromolecules of animal cells in culture have strengthened suggestions that changes in membrane permeability play an important role in controlling protein, RNA, and DNA synthesis. For example, when contact-inhibited cells are initiated to divide by the addition of fresh serum, there is a very rapid increase in the transport of phosphate and uridine (6, 31, 32). Since the morphological changes in CMV-infected cells are significant, we considered the possibility that changes in membrane structure brought about the induc-

tion of cellular RNA synthesis in CMV-infected cells. However, the measurement of trichloroacetic-soluble acid and -insoluble radioactivity in CMV-infected and -uninfected cultures labeled with [^{3}H]uridine in this study (Table 3) revealed that the increment of uridine transport was about 50% at 40 h PI, whereas that of RNA synthesis augumented four times that of uninfected cultures. In fact, the increased rate of RNA synthesis was threefold higher in CMV-infected cells than in uninfected cells where no difference of uridine transport was observed 24 h PI. In addition, it was found that the inhibitory effect of cycloheximide on uridine transport was only 10%, whereas that on RNA synthesis was approximately 60% as compared to untreated cells. These findings indicated that CMV-induced cellular RNA synthesis may not be controlled by the level of nucleotide precursors available for synthesis. The method used for dtermining pool size does not exclude the possibility that CMV infection increases specific incorporation of radio-labeled uridine into RNA, and there we are currently analyzing in detail the rates of RNA synthesis and the specific activity of uridine nucleotide pool size in CMV-infected and -uninfected cells.

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LITERATURE CITED

1. Aurelian, L., and B. Roizman. 1965. Abortive infection of canine cells by herpes simplex virus. II. The alternative suppression of synthesis of interferon and viral constituent. J. Mol. Biol. **11**:539–548.
2. Azume, M., J. R. Hodgson, C. Aizawa, and H. W. Fisher. 1969. New nuclear ribonucleic acid in cell cultures infected with polyoma virus. J. Virol. **3**:275–277.
3. Baseman, J. B., D. Paolini, Jr., and H. Amos. 1974. Stimulation by insulin of RNA synthesis in chick fibroblasts. J. Cell Biol. **60**:54–64.
4. Ben-Porat, T., T. Rakusanova, and A. S. Kaplan. 1971. Early functions of the genome of the herpesvirus. II. Inhibition of the formation of cell-specific polysomes. Virology **46**:890–899.
5. Costanzo, F., L. Fiume, M. LaPlaca, A. Mannini-Palenzona, F. Novello, and F. Strips. 1970. Ribonucleic acid polymerase induced by vaccinia virus: lack of inhibition by rifampicin and α-amanitin. J. Virol. **5**:266–269.
6. Cunningham, D. D., and A. B. Pardee. 1969. Transport changes rapidly initiated by serum addition to "contact inhibited" 3T3 cells. Proc. Nat. Acad. Sci. U.S.A. **64**:1049–1056.
7. Dahl, R., and J. R. Kates. 1970. Synthesis of vaccinia virus "early" and "late" messenger RNA in vitro with nucleoprotein structures isolated from infected cells. Virology **42**:463–472.
8. Dinowitz, M., and M. Green. 1972. The effect of infection with adenovirus 2 on the transcription of cellular RNA. Virology **50**:619–629.
9. Fioretti, A., T. Furukawa, D. Santoli, and S. Plotkin. 1973. Nonproductive infection of guinea pig cells with human cytomegalovirus. J. Virol. **11**:998–1003.
10. Flanagan, J. F. 1967. Virus specific ribonucleic acid synthesis in K. B. cells infected with herpes simplex virus. J. Virol. **1**:583–590.
11. Furukawa, T., A. Fioretti, and S. Plotkin. 1973. Growth characteristics of cytomegalovirus in human fibroblasts with demonstration of protein synthesis early in viral replication. J. Virol. **11**:991–997.
12. Gillespie, D., and S. Spiegelman. 1965. A quantitative assay for DNA-RNA hybrids with DNA immobilized on a membrane. J. Mol. Biol. **12**:829–842.
13. Hay, J., G. J. Koteles, H. M. Keit, and H. Subak-Sharpe. 1966. Herpes virus specified ribonucleic acids. Nature (London) **210**:387–390.
14. Huang, E. S., S. T. Chen, and J. S. Pagano. 1973. Human cytomegalovirus. I. Purification and characterization of viral DNA. J. Virol. **12**:1473–1481.
15. Kaplan, A. S. 1973. A brief review of biochemistry of herpesvirus host-cell interaction. Cancer Res. **33**:1393–1398.
16. Ledinco, N. 1970. Transient stimulation of deoxyribonucleic acid-dependent ribonucleic acid polymerase and histone acetylation in human embryonic kidney cultures infected with adenovirus 2 or 12: apparent induction of host ribonucleic acid synthesis. J. Virol. **6**:58–68.
17. Manialis, G. M., R. A. Rifkind, A. Bank, and P. A. Marks. 1973. Early stimulation of RNA synthesis by erythropoietin in cultures of erythroid precursor cells. Proc. Nat. Acad. Sci. U.S.A. **70**:3189–3194.
18. Moss, B., E. N. Rosenblum, E. Katz, and P. M. Grimley. 1969. Rifampicin: a specific inhibitor of vaccinia virus assembly. Nature (London) **224**:1280–1284.
19. Oda, K., and R. Dulbecco. 1968. Induction of cellular m-RNA synthesis in BSC-1 cells infected by SV-40. Virology **35**:439–444.
20. Pogo, B. G. T. 1971. Biogenesis of vaccinia: effect of rifampin on transcription. Virology **44**:576–581.
21. Pogo, B. G. T., V. G. Allfrey, and A. F. Mirsky. 1966. RNA synthesis and histone acetylation during the course of gene activation in lymphocytes. Proc. Nat. Acad. Sci. U.S.A. **55**:805–812.
22. Raska, K., Jr., W. A. Strohl, J. A. Holowczak, and J. Zimmerman. 1971. The response of BHK-21 cells to infection with type 12 adenovirus. V. Stimulation of cellular RNA synthesis and evidence for transcription of viral genome. Virology **44**:296–306.
23. Rubin, A. D., and H. L. Cooper. 1965. Evolving patterns of RNA metabolism during transition from resting state to active growth in lymphocytes stimulated by phytohemagglutinin. Proc. Nat. Acad. Sci. U.S.A. **54**:469–476.
24. St. Jeor, S. C., T. B. Albrecht, F. D. Funk, and F. Rapp. 1974. Stimulation of cellular DNA synthesis by human cytomegalovirus. J. Virol. **13**:353–362.
25. Sydiskis, R. J., and B. Roizman. 1966. Polysomes and protein synthesis in cells infected with a DNA virus. Science **153**:76–78.
26. Tamara, R., I. Ben-Porat, and A. S. Kaplan. 1972. Effect of herpes virus infection on the synthesis of cell specific-RNA. Virology **49**:537–548.
27. Tata, J. R. 1961. Hormones and the synthesis and utilization of ribonucleic acids. Prog. Nucleic Acid Res. Mol. Biol. **5**:191.
28. Todaro, G. J., G. K. Lazar, and H. Green. 1965. Quantitative studies of the growth of mouse embryo cells in culture and their development into established lines. J. Cell Biol. **17**:299–313.
29. Todaro, G. J., Y. Matsuya, S. Bloom, A. Robbins, and H. Green. 1967. Stimulation of RNA synthesis and cell

division in resting cells by a factor present in serum, p. 87–102. *In* V. Defendi and M. Stocker (ed.), Growth regulating substances for animal cells in culture. The Wistar Inst. Symp. Monogr. No. 7. The Wistar Institute Press, Philadelphia.

30. Wagner, E. M., and B. Roizman. 1969. Ribonucleic acid synthesis in cells infected with herpes simplex virus. I. Pattern of ribonucleic acid synthesis in productively infected cells. J. Virol. **4**:36–46.
31. Weber, M. J., and G. Edlin. 1971. Phosphate transport, nucleotide pool, and ribonucleic acid synthesis in growing and in density-inhibited 3T3-cells. J. Biol. Chem. **246**:1828–1833.
32. Weber, M. J., and H. Rubin. 1970. Uridine transport and RNA synthesis in growing and in density-inhibited animal cells. J. Cell Physiol. **77**:157–168.