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Effect of Adenovirus on Metabolism of Specific Host mRNAs: Transport Control and Specific Translational Discrimination

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We have studied the adenovirus-induced inhibition of host cell protein synthesis and the effect of infection on the overall metabolism of host cell mRNA during the late phase of adenovirus infection by following the fate of a number of cellular mRNAs complementary to specific cloned DNA segments. At a time in infection when the rate of total cellular protein synthesis is drastically (>90%) reduced, transcription of specific cellular genes is undiminished. However, the transport of newly synthesized cellular mRNA to the cytoplasm is greatly decreased. This decreased appearance of new mRNA in the cytoplasm cannot account for the observed cessation of cell specific protein synthesis, however, since the concentration of several preexisting cellular mRNAs, including the mRNA for actin, remains unchanged throughout the course of infection. The preexisting mRNA is intact, capped, and functional as judged by its ability to direct protein synthesis in vitro in a cap-dependent fashion. The interruption in host translation appears to operate at the level of initiation directly, since we find that fewer ribosomes are associated with a given cellular mRNA after infection than before infection. Furthermore, the in vivo inhibition of cellular protein synthesis does not appear to be the result of competition with viral mRNA, since conditions which prevent the efficient initiation of translation of viral mRNA (infection with a viral mutant) do not result in the recovery of cell translation. Thus, it appears that a late adenovirus gene product directly mediates a shutoff of host protein synthesis.

The interaction of a virus with its host cell often leads to an alteration in the synthesis and metabolism of macromolecules in the cell. The study of these alterations offers the opportunity to gain information concerning the normal course of events within the cell. For example, many different cytolytic virus infections result in decreased host protein synthesis at the same time that viral protein synthesis increases (22). The molecular basis for this viral "takeover" is best understood for poliovirus (9, 19, 39, 42) and reovirus (33) infections, where differentiation between capped and uncapped mRNAs allows discrimination between viral and host mRNAs. However, the long-standing observation that host protein synthesis is greatly depressed late in adenovirus infection (2, 4, 5) cannot be easily explained; adenovirus mRNAs are all capped (12, 23, 35, 45) and polyadenylated [poly(A)$^+$] (28) and therefore would appear to be indistinguishable from host mRNAs on a gross level (6, 32). Virtually all of the newly labeled mRNA that reaches the polyribosomes is virus specific (5, 20, 27), suggesting that transcription of cellular genes may be inhibited. However, neither the rate of synthesis of specific cellular mRNAs nor the fate of preexisting cellular mRNA has been examined with cloned cell DNA sequences.

Beltz and Flint (5) approached the question of host cell mRNA manufacture and host protein synthesis by using cDNA hybridization to total cellular mRNA. They concluded that whereas nuclear RNA complementary to the host cDNA continued to be formed and polyadenylated, no newly labeled host cell mRNA appeared in the cytoplasm. They suggested that this deprivation of host cell mRNA coupled with the massive influx of viral mRNA was probably responsible for the declining host cell protein synthesis.

However, these studies only determined that the overall transcription rate was unchanged after subtraction of the contribution from viral and rRNA transcription. The analysis thus left unclear the question of the actual transcription rate of specific mRNA-encoding genes. It did seem likely from these results that newly synthesized host cell RNA was not accumulating as stable mRNA in the cytoplasm late in infection. But whether this was due to a specific defect in transport or rather to transcription or even rapid

turnover of mRNA as it entered the cytoplasm was not fully answered. Furthermore, whether this shutdown of appearance of new mRNA in the cytoplasm was sufficient to account for translational control was also not answered. For instance, if the cellular mRNAs were long-lived, then an inhibition of transport might not have an impact on the steady-state population of preexisting cytoplasmic mRNA.

We have therefore examined these questions by using clones of specific HeLa cell mRNAs so as to measure directly transcription, transport, and RNA stability during late adenovirus infection. Our results indicate that adenovirus does in fact block the appearance of cellular mRNA in the cytoplasm while the genes continue to be transcribed. However, despite the fact that no newly synthesized mRNA appears in the cytoplasm, there is little or no change in the steady-state amount of the various mRNAs at a time when protein synthesis is inhibited by greater than 90%. Indeed, specific translation control in the form of discrimination between viral and cellular mRNA appears to be responsible for the shutoff of host protein synthesis.

MATERIALS AND METHODS

Cells and viruses. HeLa cells were grown in Joklik modified minimal essential medium (GIBCO Laboratories, Grand Island, N.Y.) containing 5% fetal calf serum (Sterile Systems Inc.). Wild-type adenovirus type 2 and mutants *dl*331 and H5*ts*125 were grown in HeLa cells and purified by methods previously described (24).

cDNA synthesis and recombinant DNA procedures. cDNA molecules were synthesized from HeLa cell cytoplasmic poly(A)$^+$ mRNA, using the avian myeloblastosis virus reverse transcriptase (supplied by J. W. Beard, Life Sciences, Inc.) as described by Land et al. (17). Double-stranded cDNA was tailed with deoxycytidylic acid residues and annealed to pBR322 cut with *Pst*I and then tailed with deoxyguanylic acid residues. The annealed vector was used to transform *Escherichia coli* LE392, and the transformed bacteria were spread directy onto L-broth agar plates containing 40 μg of thymidine and 25 μg of tetracycline per ml and incubated at 37°C. Tetracycline-resistant colonies were screened by using ^{32}P-labeled cDNA reverse-transcribed from HeLa poly(A)$^+$ cytoplasmic RNA. Colonies that gave the strongest hybridization signal were grown and further screened for the ability of plasmid DNA to hybridize [^{3}H]uridine-labeled HeLa cell poly(A)$^+$ RNA. The colonies used in this study had a single insert as determined by *Pst*I digestion of plasmid DNA and detected a single band in a Northern gel of HeLa cytoplasmic A$^+$ RNA.

RNA labeling and purification. Procedures for the isolation of both cytoplasmic and nuclear RNA have been described previously (14, 24, 34) and are briefly outlined in the legend to Fig. 3. For the continuous [^{3}H]uridine labeling experiment (see Fig. 2), unlabeled uridine was added to give a final concentration of 14 μM to prevent depletion of uridine during the course of the experiment. Poly(A)$^+$ RNA was isolated as described by Jelinek and Leinwand (14).

RNA-DNA hybridization. The transfer of RNA from agarose-formaldehyde gels to nitrocellulose was performed as described by Thomas (37). Filters containing transferred RNA were prehybridized for 12 h at 45°C in 20 mM $NaPO_4$ (pH 7.4)–6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–40% formamide–0.1% sodium dodecyl sulfate (SDS)–1× Denhardt solution (8) containing 100 μg of salmon sperm DNA per ml. The filters were then hybridized with ^{32}P-labeled DNA probes under the same conditions, except in 2× SSC and 10% dextran sulfate. Hybridization was for 36 h at 45°C. The filters were washed at 65°C for 1 h each time with 2× SSC, 1× SSC, and then 0.5× SSC, dried, and autoradiographed at −70°C with Kodak XAR-5 film and intensifying screens. Nick translation of probe DNA was performed by the method of Rigby (30).

Transcription in isolated nuclei and dot hybridization. In vitro transcription in isolated nuclei was performed as described by Hofer and Darnell (13) and Weber et al. (41). The transcription assay used 1 mCi of [^{32}P]UTP (3,000 μCi/mmol), and the assay mixture was incubated for 10 to 15 min. Nuclear RNA was isolated by the hot-phenol method of Soeiro and Darnell (34) and broken by treatment with 0.1 N NaOH for 45 min on ice, before hybridization as described previously (24). For dot blots (15), 10 μg of DNA was denatured by boiling in 0.1 N NaOH for 10 min, diluted with cold 2 M NaCl, and "dotted" onto a 45-μm nitrocellulose filter.

In vitro translation and SDS-polyacrylamide gel electrophoresis. Cytoplasmic RNA to be translated was phenol extracted and selected on polyuridylate-Sepharose. Equal amounts of poly(A)$^+$ mRNA from infected and uninfected cells (as determined by Northern blots, using an actin probe) were added to reticulocyte extracts (Bethesda Research Laboratories) at subsaturating levels (determined beforehand). Translation was for 1.5 h at 30°C, and samples were then boiled in 3% SDS–0.7 M beta-mercaptoethanol for 5 min. In vitro- and in vivo-labeled samples were electrophoresed in a 10% acrylamide gel (16). The gels were then fixed in 7% acetic acid–50% methanol, treated with En^{3}Hance (New England Nuclear Corp.), and fluorographed at −70°C.

RESULTS

Preparation and utilization of HeLa cell cDNA clones. Previous studies which attempted to understand the effects of adenovirus infection on host cell RNA metabolism have relied on measurements of total host cell RNA. It is not surprising, therefore, to find certain disagreements among these previous reports. Green et al. (10) and Price and Penman (29) reported that host cell RNA synthesis continues late in adenovirus infection, based on the observation that only ~14 to 18% of newly synthesized nuclear RNA was complementary to viral DNA. Philipson et al. (27), in contrast, found 40% of newly synthesized nuclear RNA and 70 to 80% of the poly(A)$^+$ nuclear RNA to be viral, and they therefore concluded that cellular heterogeneous

nuclear RNA synthesis production was severely depressed. Beltz and Flint (5) used a cDNA probe against total cellular RNA to show that, overall, host RNA synthesis continued. They also observed that cellular mRNA in the cytoplasm failed to become labeled after a 2-h [^{3}H]uridine pulse, which they interpreted as a reduced rate of transport of host cell RNA. Philipson et al. (27) reported that late in infection the host cell mRNA is stable in the cytoplasm and, thus, that lack of host mRNA stability was not the cause of a reduced rate of host cell protein synthesis. However, this measurement utilized a pulse-chase taken only to 10 h postinfection, i.e., merely to the beginning of the late phase of infection. Clearly, an analysis of specific sequences is required to resolve the various aspects of the effect of adenovirus infection on the transcription and metabolism of host cell mRNA. To accomplish this, therefore, we have prepared a set of cDNA clones representing moderately abundant HeLa cell mRNA.

Poly(A)$^+$ mRNA from the cytoplasm of uninfected HeLa cells was prepared, and cDNA clones were generated in the *E. coli* plasmid pBR322 by adding homopolymer (dG · dC) tails according to the procedure of Villa-Komaroff et al. (40). Clones were screened with labeled cDNA to identify clones containing moderately abundant to abundant mRNA sequences. About 20 such clones were selected and propagated, and the recombinant DNA was examined to verify the separate identity of the clones used in this study (see Materials and Methods).

Host specific nuclear RNA synthesis late in infection. To measure the formation of primary transcripts from individual host cell transcription units, each of which contributes only a small proportion of total RNA synthesis, we used a method which allows the labeling of nascent RNA to high specific activity, namely, in vitro labeling of heterogeneous nuclear RNA in isolated nuclei and subsequent detection of specific hybridized RNA sequences by the dot blot method of Kafatos et al. (15). It has previously been shown for a number of different transcription units that isolated nuclei faithfully elongate the already-initiated nascent RNA when incubated in vitro in the presence of nucleoside triphosphates (7). New RNA chains are initiated poorly, if at all, thus allowing a measurement of the differential transcription rate between various transcription units at the time the nuclei were prepared. The dot hybridization technique carried out with excess unlabeled DNA hybridized with ^{32}P-labeled nascent nuclear RNA is capable of detecting 10 counts above background on a 3-mm dot within 24 to 48 h of autoradiographic exposure (M. Salditt-Georgieff and J. E. Darnell, unpublished data). With this technique we found that the transcription rates for the five HeLa cDNA clones that were tested were the same in cells late in adenovirus infection as in uninfected HeLa cells (Fig. 1).

Lack of appearance of new host cell mRNA in the cytoplasm of infected cells. A number of earlier experiments have indicated that the majority of the labeled RNA that reaches the polyribosomes of cells late in infection is complementary to viral DNA (5, 20), but in none of these experiments was host cell mRNA measured directly. We therefore determined the time course of cytoplasmic appearance of labeled mRNA complementary to each of eight HeLa cell cDNA clones, of which four are shown in Fig. 2. It is apparent that in the uninfected cells, labeled mRNA accumulated at an approximately linear rate during the first 4 h of labeling (Fig. 2) (an indication of a half-life of at least several hours and probably longer) but that in the infected cells, little or no labeled RNA was detectable in the cytoplasm.

Therefore, from these results it would appear that in adenovirus-infected cells transcription of several specific genes continues at normal rates but that this transcription contributes little or no RNA to the cytoplasm. This result would indicate that either there is rapid nuclear degradation of cellular RNA or its transport into the cytoplasm is impaired. A third possibility, that RNA is rapidly turned over upon appearing in the cytoplasm, is very unlikely: if the failure to accumulate labeled mRNA were due to a difference in the cytoplasmic half-life of cellular mRNA in infected compared with uninfected cells, it would have to be a very large difference, since even viral mRNAs with half-lives of 30 min or less show a linear increase in labeled mRNA for the first 90 min of labeling (3, 43). However, Fig. 2 shows an apparent failure of appearance even during the first hour of labeling, for each of the cellular mRNAs in infected cells. In addition

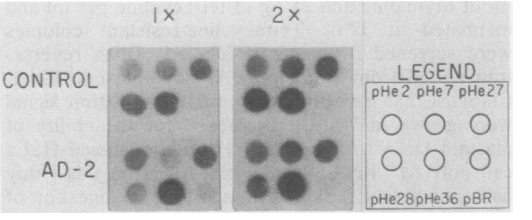


FIG. 1. Dot blot hybridization of RNA labeled in nuclei isolated from adenovirus type 2 (AD-2)-infected or mock-infected HeLa cells. Nuclear RNA of 15-h-infected or mock-infected cells (3×10^8 of each) was labeled in vitro with [^{32}P]UTP as described in the text, and 2.1×10^7 cpm (1×) or 4.3×10^7 cpm (2×) was hybridized to a dot blot containing 10 μg of each of five HeLa cDNA clones (pHe's) and pBR322 DNA (pBR).

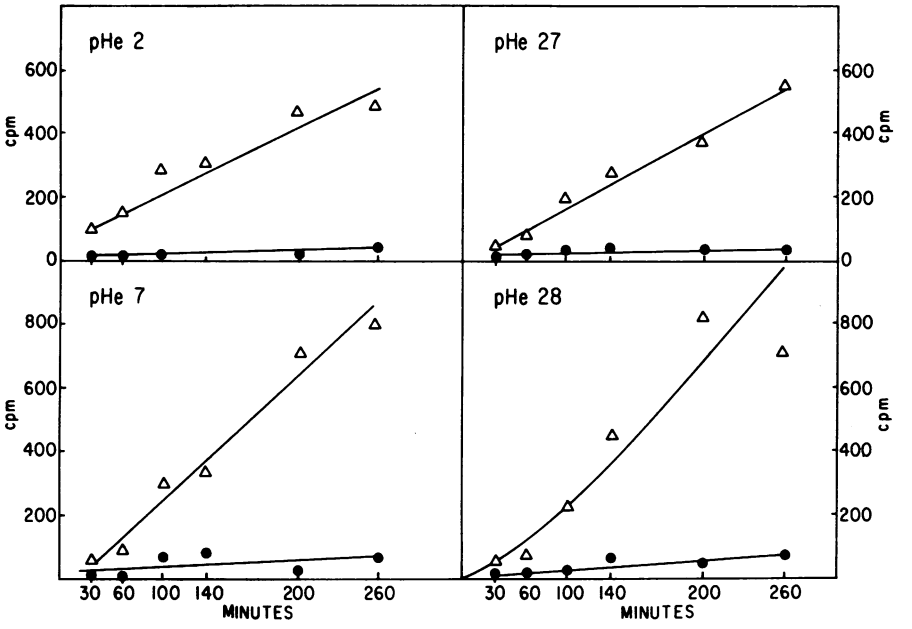


FIG. 2. Cytoplasmic accumulation of specific HeLa mRNAs in adenovirus type 2-infected (●) or uninfected (△) HeLa cells. At 15 h after infection, both the infected and the mock-infected cultures were concentrated to a density of 2×10^6 cells per ml in fresh minimal essential medium, and [^{3}H]uridine was added to a concentration of 200 μCi/ml and 14 μM. At the times indicated, 4×10^7 cells were removed from each culture, and poly(A)$^+$ cytoplasmic RNA was prepared and then hybridized to filters bearing plasmid DNA specific for a different HeLa mRNA. The results obtained with four of these HeLa cDNA clones are presented.

(see below), the preexisting cellular mRNAs are not subject to specific degradation. Thus, it seems most likely that very little of the newly synthesized cellular mRNA is delivered to the cytoplasm during late adenovirus infection.

Because the transcription units corresponding to the HeLa cell cDNA clones produced RNA at such low rates, it was not possible to observe labeled nuclear RNA precursors directly and therefore to determine whether nuclear processing was affected by infection. Moreover when the steady-state poly(A)$^+$ nuclear RNA was examined by Northern blot analysis (electrophoresis, transfer to nitrocellulose, and hybridization to nick-translated DNA probes), the only bands observed were the same size as the cytoplasmic poly(A)$^+$ molecules discussed below. We believe that these molecules do not represent cytoplasmic contamination of the nuclear fraction because under identical extraction conditions, several viral mRNAs which are abundant in the cytoplasmic fraction are not even detectable in the nuclear fraction, indicating that cytoplasmic contamination of the nuclear fraction is not significant under the conditions used (Babich and Nevins, unpublished data). These nuclear molecules could represent continued processing in the nucleus of host mRNA, but without being able to follow specific precursors, it was not possible to determine whether nuclear RNA processing is continued. Therefore, the failure of labeled cellular mRNA to appear in the cytoplasm could be due to failure of nuclear processing or specific failure in the transport of cellular mRNA. It does not appear to be due to massive degradation of cellular RNA in the nucleus, however.

Concentration of host cell mRNAs in infected cells. Since many cellular mRNAs are long-lived (11) (Fig. 2), and since there is no evidence for an effect on the metabolism of cellular mRNAs during the early phase of viral infection (3, 27), the cytoplasm of infected cells might, at the onset of the late phase of infection, contain a full complement of cellular mRNAs regardless of an effect due to failure of delivery of new mRNA into the cytoplasm. This actually is the case (Fig. 3). The concentration of cellular mRNA at 15 h after infection was examined by Northern blot analysis to detect the presence and approximate amounts of specific cellular mRNA species in infected cells. The results for four different cloned species are shown. In each case an mRNA band of identical size and approximately equal abundance was observed in both uninfected-cell mRNA and in RNA from cells infected for 18 h. Although adenovirus infection interrupts the delivery of newly formed host mRNA to the cytoplasm (Fig. 2), the preexisting cellular mRNA survives. The block in transport has little

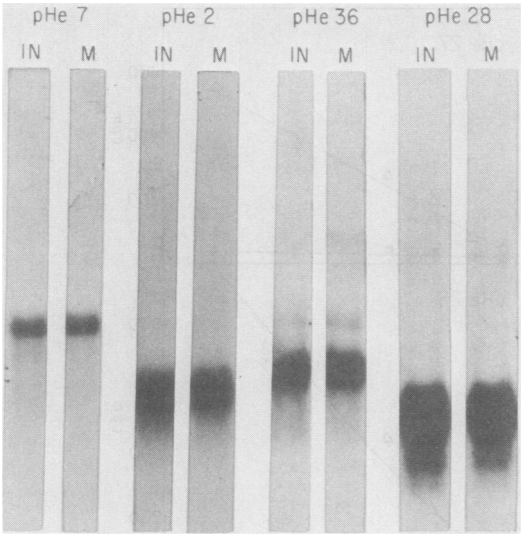


FIG. 3. Cytoplasmic concentrations of four different HeLa cell mRNAs in late-adenovirus-infected (IN) and uninfected (M) cells. Poly(A)$^+$ cytoplasmic RNA from approximately 5×10^7 cells was subjected to electrophoresis in a 1.5% agarose-formaldehyde gel and transferred to nitrocellulose. The nitrocellulose filter was then hybridized with ^{32}P-labeled plasmids containing the HeLa cell cDNA sequences indicated. (All samples were not run at same time; sizes were estimated by comparison with rRNA markers and with the adenovirus protein IX mRNA [1]). The estimated sizes (in kilobases) were: pHe 2, 0.70; pHe 7, 1.05; pHe 28, 0.62; pHe 36, 0.75.

impact on the total preexisting cellular RNA and hence cannot alone account for the block in protein synthesis.

At this time of infection (15 h), there is a drastic reduction in host cell protein synthesis (2, 4, 5) (Fig. 4). Thus, since there were normal levels of specific HeLa mRNAs present in the cytoplasm at the time when HeLa protein synthesis was completely shut off, it seemed most likely either that there was a specific translational control mediated by the virus that favored the translation of late viral mRNA or that the host cell mRNA was rendered nontranslatable through some subtle modification. To establish whether the depression of host protein synthesis was due to the lack of integrity of cellular mRNA, the translatability of cellular mRNA in vivo and in vitro was compared. First, normal and infected cells were pulse-labeled with [^{35}S]methionine at 15 h postinfection, and labeled proteins were analyzed by polyacrylamide gel electrophoresis and fluorography. Poly(A)$^+$ RNA from an equal amount of the same culture was also prepared. A portion of this RNA was translated in vitro, and an additional portion was utilized for a Northern blot to assay for the concentration of a specific mRNA in the two samples. The results of this experiment are presented in Fig. 5. There were equal amounts of actin mRNA (assayed by Northern blot analysis) in infected and uninfected cells (Fig. 5A). The actin mRNA as well as other cell mRNAs in the infected cell was actually functionally intact (Fig. 5B). Translation in vitro of the uninfected-cell mRNA and of mRNA from infected cells revealed that many cell proteins, including actin,

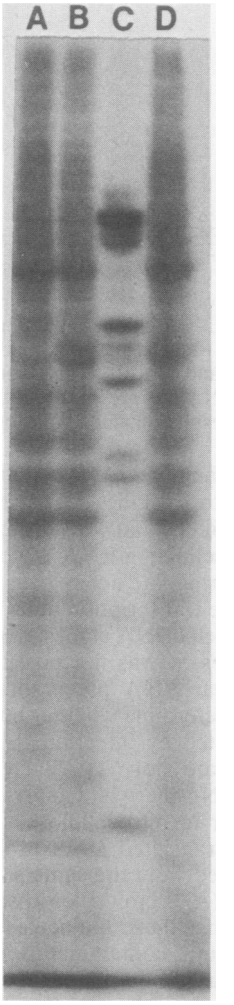


FIG. 4. Effect of adenovirus infection on host cell protein synthesis, showing requirement of a late viral function. HeLa cells were mock infected (lane A), infected with wild-type adenovirus type 5 alone (lane C) or in the presence of cytosine arabinoside (lane B), or infected with mutant H5*ts*125 (lane D). At 14 h postinfection (at 40°C) cells were labeled with [^{35}S]methionine for 30 min. The labeled proteins were then analyzed by electrophoresis in a 10% acrylamide-SDS gel followed by fluorography.

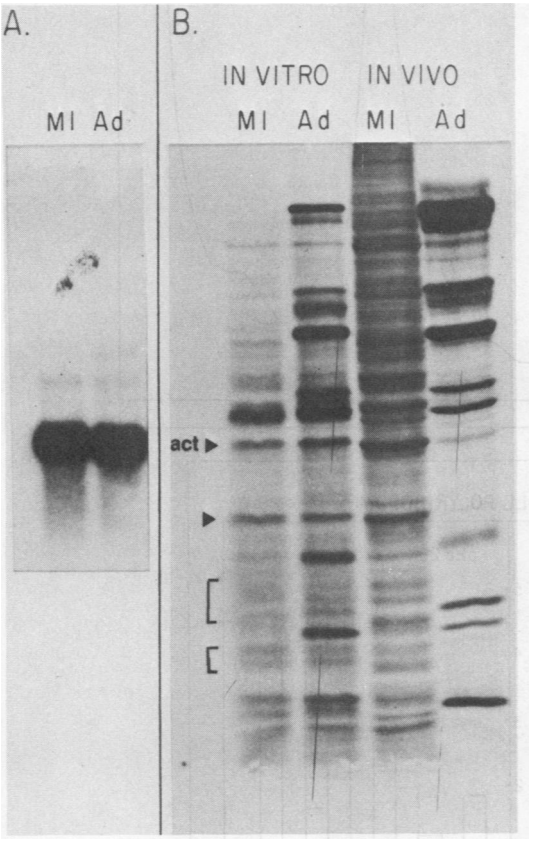


FIG. 5. In vitro and in vivo translation. (A) RNA was extracted from the cytoplasm of uninfected and 15-h-infected HeLa cells. Poly(A)$^+$ RNA from each culture was subjected to electrophoresis in an agarose-formaldehyde gel, blotted to nitrocellulose, and hybridized with a ^{32}P-labeled actin (act)-specific probe. (B) A portion of each RNA sample used for the analysis in (A) was translated in vitro in a rabbit reticulocyte lysate system with [^{35}S]methionine as the label. For in vivo labeling, the same cells were pulsed for 1 h with 20 μCi of [^{35}S]methionine per ml. All protein extracts were prepared for SDS-acrylamide gel electrophoresis and autoradiography as described in the text. MI, Mock infected; Ad, adenovirus infected.

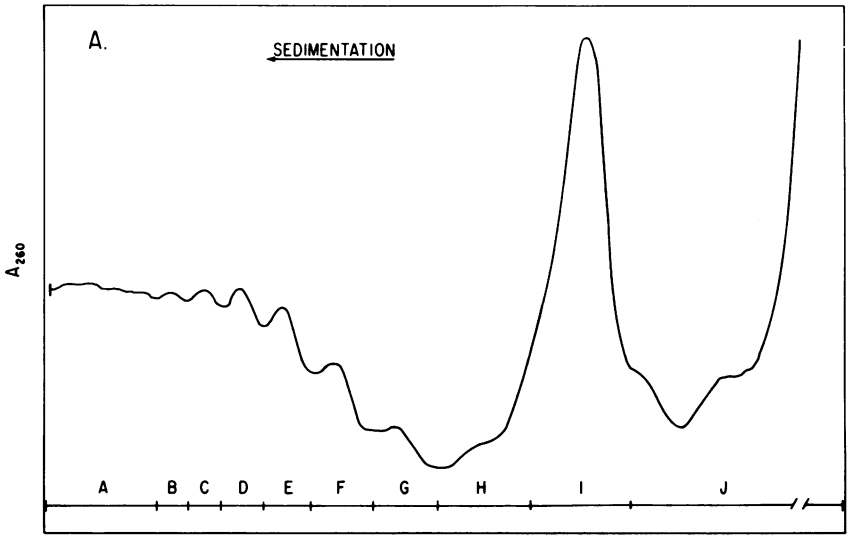
could be detected in the in vitro-labeled protein (Fig. 5B). Furthermore, the translation of each RNA sample was cap dependent as indicated by the near-complete inhibition of synthesis of specific proteins by 1 mM 7mGp (data not shown). These results are in sharp contrast to the translation of these mRNAs inside the cell; the synthesis of most of the cell proteins, including actin, was inhibited by greater than 90% (Fig. 5B).

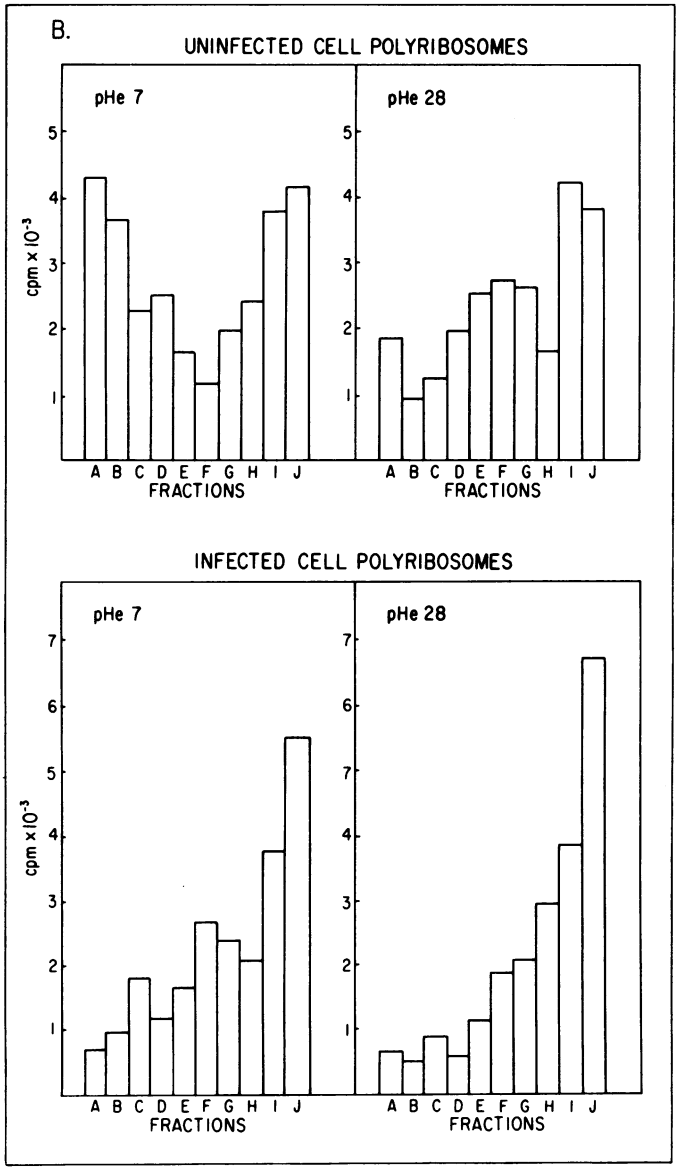
Thus, cellular mRNA must be subject to some form of direct translational inhibition in vivo. Analysis of the distribution of mRNA in polyribosomes can often reveal the level at which translational control is operating (for example, initiation or translocation). In poliovirus-infected cells, cellular protein synthesis is blocked at the level of initiation of translation, and this is manifest as fewer ribosomes per cellular message when the polysome profiles of poliovirus-infected cells are examined (19). We therefore examined the association of the preexisting host cell mRNA with ribosomes by collecting RNA from polyribosomes of various sizes and examining this RNA by Northern blot analyses for its content of specific HeLa cell mRNA sequences. The results indicated that HeLa cell mRNA was associated with ribosomes in cells infected for 15 h but that there was a consistent shift to association with fewer ribosomes in infected compared with uninfected cells (Fig. 6). For example, sequences complementary to clone 7 were prominent in polysomes with six to eight ribosomes in uninfected cells but were associated with only two to three ribosomes after infection. If all other aspects of protein synthesis are normal, but initiation is deficient, the result is fewer ribosomes per mRNA. Thus, decreased initiation of host mRNA is probably responsible for the observed results.

Finally, an experiment was carried out to investigate whether the inhibition of host mRNA translation was simply due to displacement of cellular mRNA from ribosomes by the large amounts of virus-specific mRNA present late in infection or, rather, due to a specific discrimination event. Cells were infected with *dl*331, an adenovirus mutant lacking the VA 1 sequences (36). Cells infected with this mutant produce late adenovirus mRNA in wild-type amounts but translate the viral mRNA considerably less well than normal (36). This result was confirmed (Fig. 7, lanes VA and Ad), but it was also obvious (Fig. 7, lanes MI and VA) that host protein synthesis nevertheless declines. (Translation in vitro of the RNA from *dl*331-infected cells showed that host mRNA was still present [data not shown].) Thus, despite the fact that the late viral mRNA is translated poorly, the cellular mRNA is still not translated in adenovirus *dl*331-infected cells. We therefore conclude that the adenovirus-mediated shutoff of host cell protein synthesis is the result of a specific translational control that discriminates between viral and cellular mRNA.

DISCUSSION

The principal conclusion from the results in this paper is that adenovirus infection results in a specific inhibition of translation of preexisting host cell mRNA molecules but that mRNAs are still present in the infected cell and can be translated in vitro. This and other conclusions discussed below are based on the study of


A.
SEDIMENTATION
A_{260}
A B C D E F G H I J


B.
UNINFECTED CELL POLYRIBOSOMES
pHe 7
pHe 28
cpm x 10^{-3}
1 2 3 4 5
A B C D E F G H I J
FRACTIONS
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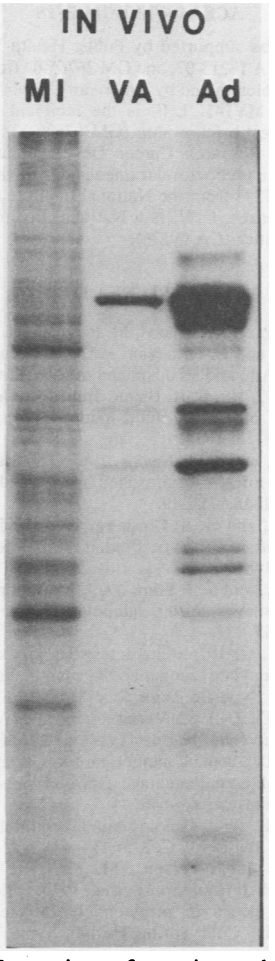

FIG. 7. Comparison of protein synthesis in uninfected cells, cells infected with adenovirus type 5, and cells infected with mutant *dl*331 (the VA mutant) at 15 h after infection. HeLa cells were pulse-labeled for 1 h with 20 μCi of [^{35}S]methionine per ml, and an equal portion from each infection was prepared for SDS-polyacrylamide gel electrophoresis and autoradiography as described in the text. MI, Mock infected; VA, *dl*331 infected; Ad, adenovirus type 5 infected.

cellular RNA complementary to eight specific cloned DNA sequences from HeLa cells.

The major factor in the shutoff of host protein synthesis appears to be decreased initiation of protein synthesis of the preexisting cellular mRNA. Based on the data presented in this paper, we would conclude that this decrease in host cell mRNA utilization is the result of a specific effect imposed by virus infection. First, the cellular mRNA is intact and can be translated in vitro. Thus, it does not appear that the virus has caused a physical alteration of the host cell mRNAs. Second, the inhibition of translation of host cell mRNA is not due simply to a "mass action" competition imposed by the translation of the large amount of viral mRNA that is present late in infection. Cellular protein synthesis was found to be inhibited in *dl*331-infected cells to the same degree as in a wild-type infection. Under the conditions of *dl*331 infection, late viral mRNA production is normal, but the translation of these late viral mRNAs is greatly reduced (36). Thus, in the absence of translation of the supposed competitor, the translation of the host mRNA does not recover. We are then left to conclude that a late viral function must effect a discrimination between the host and viral mRNAs.

In addition to our conclusions about translation control, the use of cloned DNA sequences complementary to specific cellular mRNAs has strengthened the conclusions of Beltz and Flint (5) concerning the effect of adenovirus infection on the biogenesis of cellular mRNA. In particular, we have shown directly that the transcription of several host genes, which contribute cytoplasmic mRNA, does not change upon adenovirus infection. Also, despite continued RNA synthesis, the appearance of newly synthesized specific mRNA molecules into the cytoplasm was greatly impaired. There appears, therefore, to be a specific inhibition of the transport of cellular mRNAs during late viral infection.

However, this cessation of delivery of cellular mRNA to the cytoplasm does not explain the dramatic shutoff of cellular protein synthesis. At a time when the synthesis of most cell proteins has declined to less than 10% of normal levels, there are still normal amounts of cellular mRNAs present in the cytoplasm. Since both viral and cellular mRNAs are capped and polyadenylated, there is no ready suggestion as to how viral mRNA translation can be favored. For example, while most late mRNAs come from the major late transcription unit and therefore share leader sequences, there are mRNAs from at least three other (early) transcription units, 1A, 1B, and protein IX, that do not possess these leader sequences but are nevertheless formed in

FIG. 6. Distribution of two HeLa specific mRNAs in the polysomes of infected and uninfected HeLa cells. Cells (1.5×10^8) from cultures infected with adenovirus type 2 for 15 h or from mock-infected cultures were lysed in 0.5% Nonidet P-40, and the cytoplasmic extracts were centrifuged through 7 to 47% sucrose gradients in 0.15 M NaCl–10 mM $MgCl_2$–0.01 M Tris (pH 8.0) at 27,000 rpm for 4 h at 2°C. (A) Optical density profile at 260 nm of the infected sample. Ten fractions (A–J) were made across each gradient as indicated. (B) Poly(A)$^+$ RNA was prepared from each fraction, bound to nitrocellulose, and then hybridized to nick-translated DNA of HeLa cDNA clones pHe 7 and pHe 28 to detect HeLa specific mRNAs. Filters were counted directly.

substantial amounts and translated late in infection (38, 44). It should be noted, however, that the efficiencies of translation of these mRNAs that are present both early and late have not been directly compared. If only those mRNAs containing the tripartite leader sequences were efficiently translated late in infection, this would provide a clue as to the basis of the translation control. If the 1A, 1B, and protein IX mRNAs are equally translated, however, there would not seem to be a ready basis to distinguish several different viral mRNA 5′ ends from all types of cellular mRNA 5′ ends.

Finally, the mechanism for the selective shutoff of host mRNA transport remains obscure. It does not appear likely that the inhibition of cell protein synthesis is indirectly responsible (for instance, by removing a short-lived transport factor), since the mere inhibition of protein synthesis with inhibitors does not abolish transport of mRNA (18, 26). The results appear to suggest instead that a viral function is responsible, one which allows, as with the translation control, a discrimination in the transport of viral and cellular sequences. If true, this clearly opens an area of considerable interest with regard to the possible control of gene expression. However, such a conclusion must await a more rigorous study of the integrity of the nuclear host cell RNA, i.e., a study comparable to the one of cytoplasmic mRNA which we report here.

Prime candidates for molecules involved in both processing and transport are the proteins associated with RNA that are known to interact with the RNA even as it is being synthesized. Proteins which are associated with cytoplasmic mRNA appear to be distinct from, and not a subset of, the proteins which are associated with the RNA in the nucleus, and the movement of RNA from nucleus to cytoplasm seems to involve the exchange of the nuclear for the cytoplasmic set of these proteins (25, 31). If this protein exchange is associated or coupled with transport, then virus infection could interrupt cellular transport by limiting the concentration of these proteins on one side of the nuclear membrane or the other. The virus could circumvent this inhibition of transport by encoding a protein(s) which can specifically recognize and transport viral RNA. In this regard it is worth mentioning that the 100-kilodalton adenovirus L4 protein appears to be in association with poly(A)$^+$ ribonucleoprotein structures isolated from late-infected cells (21). The protein composition of cellular and viral ribonucleoproteins might provide an important clue as to how discrimination between viral and cellular mRNAs is made and might serve to identify proteins involved in important processing or transport functions.

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