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Inhibition of Host Protein Synthesis and Degradation of Cellular mRNAs During Infection by Influenza and Herpes Simplex Virus

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Cloned DNA copies of two cellular genes were used to monitor, by blot hybridization, the stability of particular cell mRNAs after infection by influenza virus and herpesvirus. The results indicated that the inhibition of host cell protein synthesis that accompanied infection by each virus could be explained by a reduction in the amounts of cellular mRNAs in the cytoplasm, and they suggested that this decrease was due to virus-mediated mRNA degradation.

A frequent result of virus infection in eucaryotic cells is the inhibition, often dramatic, of host cellular protein synthesis. Careful investigation of this phenomenon has led to the conclusion that there may be a number of ways in which different viruses can exert this effect, including modification of the host cell translational apparatus (25, 28), competition between virus and cellular mRNAs (16), alterations in the intracellular ionic concentration favorable to translation of virus rather than host mRNAs (3), and, for the DNA virus herpes simplex virus (HSV), degradation of preexisting cellular mRNAs (18, 20). I report here experiments designed to test the latter possibility for the RNA virus fowl plague virus (FPV), an avian influenza virus, by using cloned DNA copies of two cellular genes to analyze the stability of their mRNA transcripts by blot hybridization. After infection, a progressive decrease in the concentration of both cellular mRNA species accompanied the inhibition of host protein synthesis. This decrease was not apparently due to natural turnover of mRNA in the absence of de novo synthesis, suggesting that FPV infection caused the destruction of preexisting cellular mRNAs. Analysis of HSV infection by the same approach gave similar results, confirming previous work (18, 20) and raising the possibility that a common mechanism might be responsible for the inhibition of host protein synthesis by both viruses.

Chicken embryo fibroblasts (CEF) were mock-infected or infected with FPV, and at various times after infection the pattern of protein synthesis was monitored by [^{35}S]methionine labeling and polyacrylamide gel analysis (Fig. 1a). As has been observed before (11, 27), a progressive decrease in host protein synthesis, which was particularly obvious for a number of cell proteins (Fig. 1a, arrows) including the 43,000-molecular-weight structural protein actin, accompanied the appearance of virus-specific proteins. Inefficient synthesis of cell proteins was also evident when cytoplasmic RNA from infected cells was translated in vitro (data not shown), suggesting that cell mRNAs may be somehow inactivated during infection.

To investigate this phenomenon further, equal quantities of cytoplasmic RNA, extracted at various times after infection, were analyzed for the presence of two different cellular mRNAs by blot hybridization (Fig. 1b and c). Since the overall quantity of cellular cytoplasmic RNA is unlikely to change significantly during infection, this approach should indicate whether changes in the cytoplasmic concentration of the mRNAs have occurred. The hybridization probes were ^{32}P-labeled recombinant DNA plasmids containing sequences either of the β-actin gene from chicken brain (5) (a gift from D. Cleveland through A. Rice) or of the glyceraldehyde-3-phosphate dehydrogenase (GPD) gene from chicken muscle (17) (a gift from A. McLeod). Each probe hybridized to a single major species of RNA which was present in all the cytoplasmic samples and which presumably represented mRNA because, when the RNA preparations were fractionated by oligodeoxythymidylate-cellulose chromatography before blot analysis, hybridization occurred only with polyadenylated RNA. For both DNA probes, the extent of hybridization decreased as the infection progressed, indicating a decline in the concentration of their corresponding mRNAs in the cytoplasm. No concomitant increase was observed in the

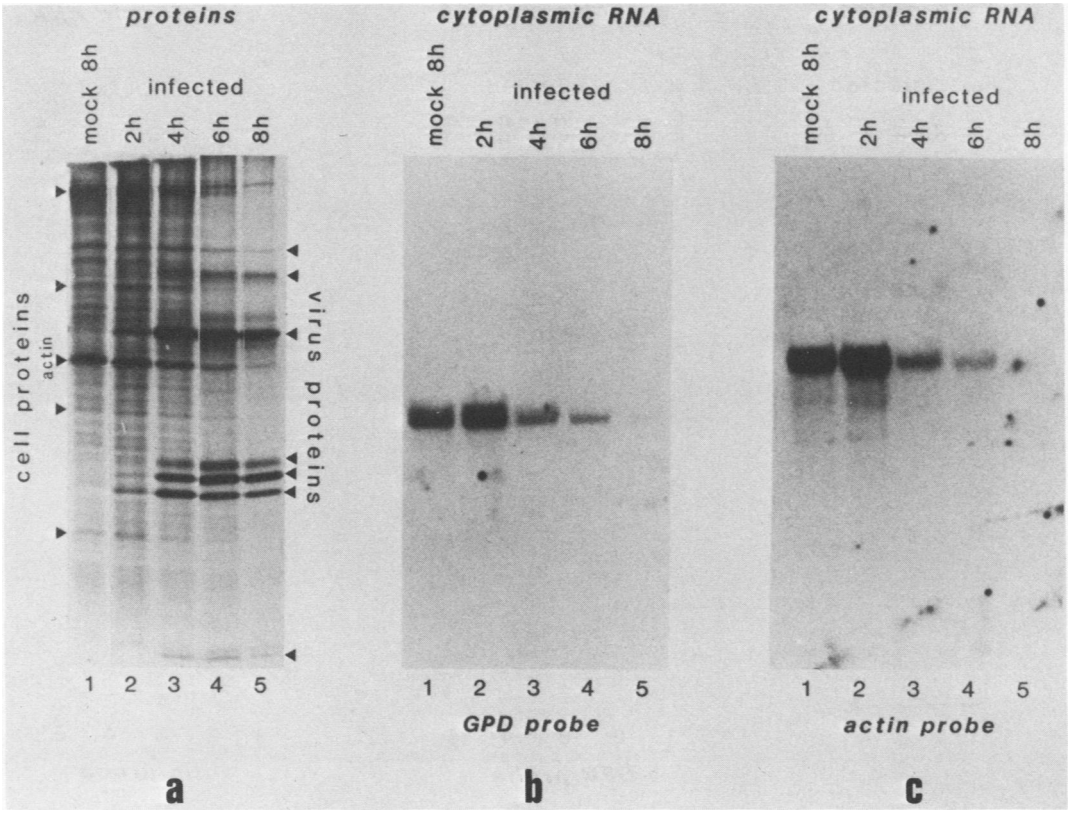

FIG. 1. (a) Polypeptides synthesized during infection of CEF cells with FPV (A/FPV/Rostock/34). Primary monolayer cultures (2) were mock-infected or infected (11) at a multiplicity of about 20 PFU/cell and were labeled for 30 min with [^{35}S]methionine (20 μCi/ml) before harvesting at the times indicated above each track. Labeled polypeptides were separated by polyacrylamide gel electrophoresis and detected by autoradiography (11). (b) Stability of GPD gene transcripts after FPV infection. Cytoplasmic RNA was extracted (12) from a further set of CEF cultures at various times after infection with FPV, and 25-μg samples were fractionated, in the order indicated above the tracks, on a formaldehyde-containing agarose gel (10). The RNAs were then transferred to a sheet of nitrocellulose by blotting procedures (31) and hybridized (10) with approximately 0.2 μg of ^{32}P-labeled plasmid DNA (pGPD1) which included a copy of the GPD gene from chicken muscle (17). The DNA probe was labeled by nick-translation (24) to a specific activity of about 5×10^7 cpm/μg. The nitrocellulose was washed to remove nonspecific radioactivity (10), and labeled bands were detected by autoradiography with preflashed film and an intensifying screen (15). (c) Stability of β-actin gene transcripts after FPV infection. The experiment was identical to that shown in (b) except that the DNA probe was a plasmid (pA1) containing sequences of the β-actin gene from chicken brain (5).

nuclei of these cells (data not shown). In this experiment, each probe hybridized less to 8-h mock-infected cell RNA than to 2-h virus-infected cell RNA (compare tracks 1 and 2 in Fig. 1b and c). A possible explanation for this is that the mock-infection procedure itself caused a reduction in the concentration of the mRNAs, but since the decrease was considerably less than was observed in 8-h virus-infected cells (Fig. 1b and c, track 5), it was still clear that virus infection accelerated the disappearance of cellular mRNAs from the cytoplasm.

A similar series of experiments was carried out with the DNA virus HSV type 1. Previous reports had indicated that HSV infection led to the degradation of preexisting globin mRNA in Friend erythroleukemia cells (18) and of virus-specific mRNAs in polyoma- or adenovirus-transformed cells (20, 29), and so it was of interest to know whether this applied also to GPD and β-actin transcripts in a more widely studied virus-cell system. The pattern of proteins synthesized in baby hamster kidney (BHK) cells at various times after infection with HSV type 1 (Fig. 2a) shows, as others have reported (8, 30), that the synthesis of cellular proteins (Fig. 2a, arrowed bands) declines throughout infection as virus-specific protein synthesis in-

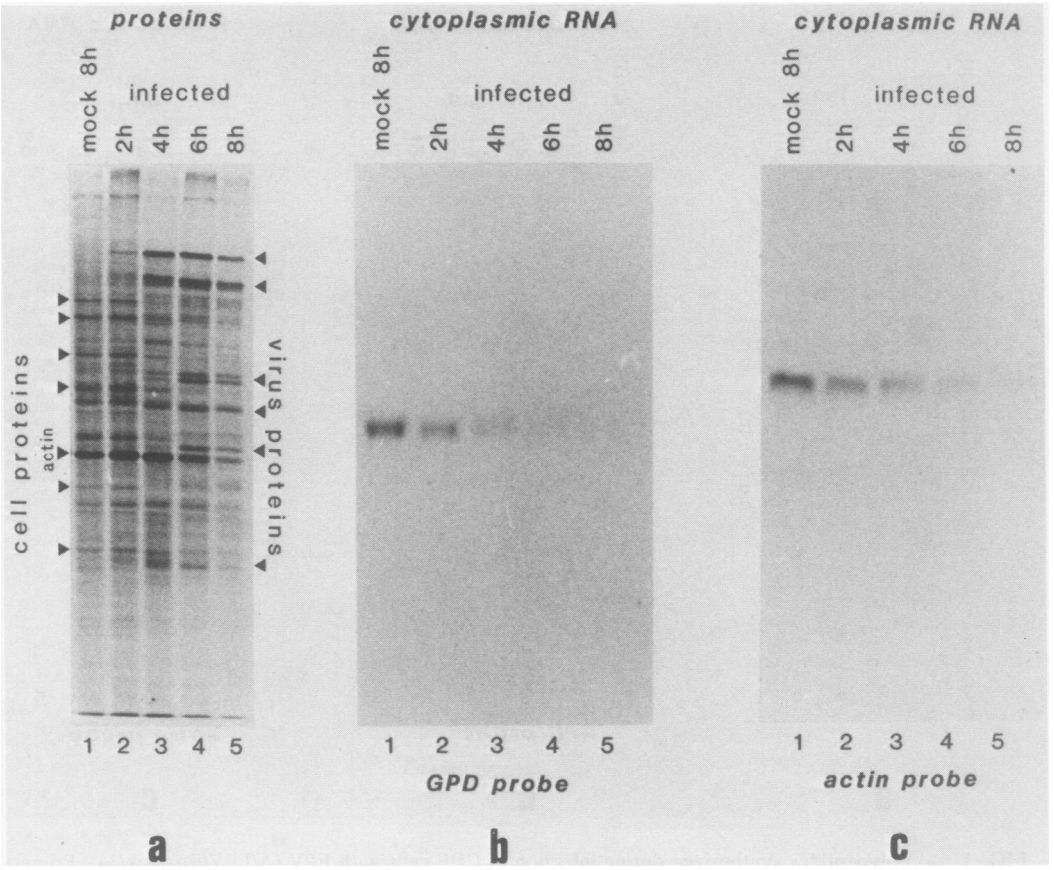

FIG. 2. (a) Polypeptides synthesized during infection of BHK cells with HSV type 1 (strain F). Monolayer cultures were mock-infected or infected at an approximate multiplicity of 10 PFU/cell, labeled with [^{35}S]methionine at various times after infection, and analyzed by gel electrophoresis as described for Fig. 1a. (b) Stability of GPD gene transcripts after HSV infection. Cytoplasmic RNA was extracted (10) from a further set of BHK cell cultures at different times after infection with HSV, and 25-μg samples were fractionated on a gel. The RNAs were transferred to nitrocellulose and hybridized with ^{32}P-labeled pGPD1 exactly as described for Figure 1b. (c) Stability of β-actin gene transcripts after HSV infection. Identical to (b) except that the DNA probe was pA1.

creases. Cytoplasmic RNA, extracted from further cultures at the same times after infection, was analyzed as described above for the presence of GPD and β-actin gene transcripts by blot hybridization (Fig. 2b and c, respectively). Though the chicken-specific probes hybridized less efficiently with the BHK cell RNA than they had with the CEF cell RNA (presumably reflecting only partial homology between the equivalent genes of the two different species), once again each detected a single major RNA species which declined in concentration throughout infection.

The reduction in concentration of cellular mRNA sequences which accompanied virus infection could be explained by natural turnover of mRNA in the absence of de novo cell transcription; both influenza virus and HSV may interfere with or modify host cell mRNA production (14, 18, 29). To test this possibility, the amounts of GPD and β-actin gene transcripts were compared in CEF and BHK cells which had been incubated for 8 h in the presence or absence of the DNA transcription inhibitors actinomycin D (7, 9) and α-amanitin (13). Cytoplasmic RNA was extracted, blotted, and hybridized as described above with ^{32}P-labeled GPD and β-actin gene probes (Fig. 3). Treatment with the drugs did not reduce greatly the concentrations of GPD transcripts in the CEF or BHK cells (tracks 1 through 3 and 7 through 9), nor of the β-actin transcripts in the BHK cells (tracks 10 through 12), but a significant reduction in β-actin RNA sequences was observed in the drug-treated CEF cells (tracks 4 through 6). However, the reduction was clearly less than that caused by

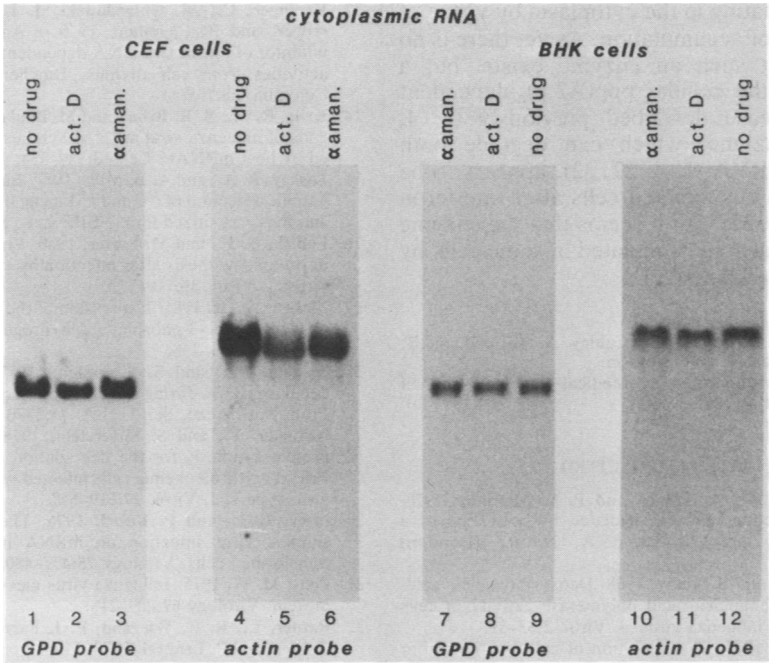

FIG. 3. Stability of GPD and β-actin gene transcripts after treatment of cells with inhibitors of DNA transcription. Cytoplasmic RNA was extracted from CEF or BHK cell cultures which had been incubated in the presence or absence of actinomycin D (10 μg/ml) or α-amanitin (20 μg/ml) for 8 h. Samples of the RNA(25 μg) were fractionated by gel electrophoresis, transferred to nitrocellulose, and hybridized with either ^{32}P-labeled pGPD1 or pA1 as described for Fig. 1.

virus infection (cf. Fig. 1b, tracks 2 and 5), suggesting that infection with each virus actively promotes degradation of preexisting GPD and β-actin mRNAs.

The results suggest that the reduction in host protein synthesis observed in cells infected with influenza virus or HSV may be a direct result of the loss from the cytoplasm of host cell mRNAs, an interpretation which is supported by the observation that during infection of CEF by another strain of influenza virus, A/PR8, in which inhibition of host protein synthesis is less obvious, the concentration of GPD and β-actin mRNA sequences declined less markedly (data not shown). It could be argued that this decrease in the amount of cellular mRNA is not the primary cause of host protein synthesis inhibition but is secondary to and perhaps a consequence of displacement of host cell mRNAs from polysomes. In this case, though, cell mRNAs should be degraded during any infection where cellular protein synthesis is suppressed, a prediction which is not borne out. Host cell mRNAs can be recovered intact (as judged by in vitro translation) from the cytoplasm of cells infected with vesicular stomatitis virus (16) at times when it is clear that their translation in vivo is inhibited. Blot hybridization experiments indicate that neither β-actin nor GPD mRNA sequences decline in concentration during poliovirus infection, though host cell protein synthesis is blocked (our unpublished results; A. Rice, personal communication). Accordingly, superinfection by poliovirus of cells already infected with vesicular stomatitis virus results in the rapid inhibition of vesicular stomatitis virus-specific protein synthesis, yet after this has occurred, functional vesicular stomatitis virus mRNAs can be purified from these cells (6).

Inactivation of host cell mRNAs through degradation could then be a common mechanism of virus-mediated inhibition of host cell protein synthesis. Indeed, cellular mRNAs are also degraded during infection with the poxvirus vaccinia virus (A. Rice, personal communication). How the inactivation might occur is not clear. Virus-encoded proteins may be involved, as has been proposed for HSV (19), but the similarity of the effects caused by such widely different viruses suggests a common factor, perhaps activation of a cellular nuclease as a response to virus infection. Virus mRNAs might be protected against the nuclease, possibly as ribonucleoprotein complexes (influenza virus mRNAs have been reported to exist as such complexes in the infected cell [21]), or might be equally suscepti-

ble, predominating in the cytoplasm by virtue of a higher rate of accumulation. As yet there is no evidence that such an enzyme exists, but a candidate is the cellular ppp(A2′p)$_n$-dependent endoribonuclease described previously (1, 4, 22). This enzyme, which can degrade both mRNA and rRNA (1, 4, 22, 32), appears to be activated in virus-infected cells after interferon treatment (23, 32), but it seems that the enzyme activity may also be stimulated in some cells by virus infection alone (26).

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