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Vaccinia Virus Induces Cellular mRNA Degradation

ANDREW P. RICE† AND BRYAN E. ROBERTS*
Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts 02115

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The infection of mouse L cells with vaccinia virus induced a rapid inhibition of cellular polypeptide synthesis and a diversion of protein synthesis to the exclusive production of viral polypeptides. This shutoff of cell-specific protein synthesis was achieved by a novel mechanism by which the virus induced the rapid degradation of cellular mRNAs. Concurrent with the degradation of cellular mRNA, the virus proceeds in the orderly temporal expression of its own genetic information. The effect of vaccinia virus infection upon two abundant L-cell mRNAs was assessed by using the highly conserved cDNA sequences that encode chicken β-actin and rat α-tubulin. Hybridization analyses demonstrated that throughout infection there is a rapid and progressive degradation of both of these mRNAs. In fact, after 3 h of infection they are reduced to less than 50% of their concentration in uninfected L cells, and between 8 to 10 h they are almost entirely degraded. This observation explains in part the mechanism by which vaccinia virus inhibits host cell protein synthesis.

Studies of both the temporal expression of viral genes during lytic infection and virus-induced perturbations of cellular macromolecular processes have provided much insight into mechanisms regulating eucaryotic gene expression. The synthesis of cellular polypeptides is often inhibited after infection, and the examination of different viruses demonstrates that this inhibition can arise from either transcriptional or post-transcriptional mechanisms. In the nucleus viral infection can subvert the maturation of cellular mRNA by impairing RNA transcription, processing, or transport; whereas in the cytoplasm, protein synthesis can be altered by directly affecting the protein synthetic apparatus, mRNA abundance, or mRNA stability.

Specific examples of alterations in host cell nuclear RNA metabolism have been documented in several viruses. Poliovirus has been shown to inactivate a factor(s) required for polymerase II transcription (12). Adenovirus inhibits the processing of cellular mRNA; subsequently, only viral mRNA is transported from the nucleus late in infection (5). Influenza virus removes the 5′ cap and 10 to 13 nucleotides from newly synthesized cellular heterogenous nuclear RNAs to prime viral transcription and produce chimeric mRNAs (18).

Viruses that replicate in the cytoplasm can directly alter components of that environment to effect changes in protein synthesis. Poliovirus and reovirus cause an alteration of the protein synthetic apparatus of the infected cell, allowing the preferential translation of uncapped viral mRNA (21, 43, 46). In contrast, vesicular stomatitis virus (VSV) simply transcribes abundant viral mRNA, which dilutes cellular mRNA and competes for limited ribosomes (27). We report here our studies of the inhibition of host cell protein synthesis during vaccinia virus infection in mouse L cells and the effects of infection on the L-cell mRNA population.

Vaccinia virus is a member of the poxvirus family; poxviruses are characterized by their large size and cytoplasmic site of replication. The vaccinia virus genome is 200 kilobase pairs with a potential coding capacity of about 180 polypeptides. It encodes many of the enzymes involved in viral DNA replication and transcription. A number of these enzymes have been purified and characterized from the vaccinia virion and include polyadenylic acid [poly(A)] polymerase, 5′ capping and methylating enzyme(s), two nucleotide triphosphate hydrolases, a single strand-specific DNase, a DNA gyrase, and a protein kinase (31).

Similar to other DNA viruses, vaccinia virus transcription occurs in two phases, "early" transcription, which does not require DNA synthesis, and "late" transcription, which is dependent upon viral DNA replication. Inhibitors of viral DNA synthesis, drugs, or *ts* viral lesions in DNA replication also inhibit the onset of viral late transcription. Purified viral core particles are capable of synthesizing early viral mRNAs

† Present address: Imperial Cancer Research Fund Laboratories, London, England.

in a cell-free system of coupled transcription-translation, but cannot synthesize late mRNAs (10, 37).

Vaccinia virus grows productively in a wide range of cell lines, including human HeLa, monkey CV-1, mouse L, and chicken CEF cells. Infection results in rapid and extensive cytopathic effects; cells become rounded and syncytial a few hours after infection (16). Concomitantly, many aspects of cellular macromolecular processes are perturbed, nuclear DNA and RNA synthesis are inhibited (4, 22), the overall amount of protein synthesis is reduced (41), and viral polypeptides are exclusively synthesized (35). Virions are assembled in the cytoplasm, where the majority remain until cell lysis. The vaccinia infectious cycle is complete in approximately 12 to 14 h.

Cooper and Moss (11) have shown the patterns of polypeptides synthesized in vivo and by in vitro translation of extracted RNA are similar throughout infection in HeLa cells. This result indicates that in infected cells there is no obvious alteration of the protein synthetic apparatus that favors the translation of vaccinia virus mRNA over that of cellular mRNA. Additionally, Boone and Moss (8) have shown in vaccinia virus-infected HeLa cells that at 2 h postinfection (p.i.) from 52 to 59% of polyadenylated [poly(A)$^+$] RNA hybridizes to vaccinia virus DNA; at 7 h p.i. from 82 to 92% of poly(A)$^+$ RNA is virus specific. These studies suggest several possibilities, including dilution of cellular mRNA by abundant viral transcription or the inactivation or degradation of cellular mRNA.

We have used cloned DNAs of β-actin mRNA (9) and an α-tubulin mRNA (25) as probes for these specific cellular mRNA sequences during vaccinia virus infection. We report here that these mRNAs are degraded during the course of infection. Degradation of cellular mRNA has also been reported in herpes simplex virus infection (33, 44).

MATERIALS AND METHODS

Cells and viruses. L cells (L 929) were obtained from Joseph Kates and were maintained as monolayer cultures in DME (GIBCO Laboratories, Grand Island, N.Y.) plus 5% calf serum (GIBCO).

Vaccinia virus (strain WR) was obtained from Joseph Kates and was twice plaque purified. Vaccinia virus growth and purification were done essentially as described by Hruby et al. (19).

Crude virus stocks were prepared by low-multiplicity passage (multiplicity of infection, 0.01 to 0.1) in L cells. To prepare stocks, cells were infected and incubated for 2 to 4 days until the cytopathic effect was complete; the cells were then harvested by centrifugation (2,000 rpm) and were suspended in 1 ml of DME (minus serum) per 2×10^7 cells. The titers of crude stocks prepared in this manner were routinely 1×10^9 to 3×10^9 PFU/ml. Crude virus stocks were dispersed before inoculation by incubation at 37°C in an equal volume of 0.25% trypsin (GIBCO).

Infections were carried out in DME plus 5% fetal calf serum (GIBCO). Cells to be infected were washed once with phosphate-buffered saline, and virus stock was added and absorbed for 30 min in 5 ml of medium per 150-mm culture dish. Additional medium was then added; this was defined as time zero.

VSV San Juan strain was obtained from Jack Rose, and the conditions of infection were those used for vaccinia virus.

In vivo labeling of cells. Cells were labeled for 15 min in medium minus methionine with [^{35}S]methionine (New England Nuclear Corp., Boston, Mass.) used at 100 μCi/ml. Labeled polypeptides were analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide electrophoresis (23) and fluorography (7).

RNA extractions. Cells were scraped from plates with a rubber policeman, harvested by centrifugation, washed twice with ice-cold phosphate-buffered saline, and quick-frozen in liquid nitrogen. The frozen pellet was taken up in lysis buffer (0.15 M NaCl, 10 mM Tris-hydrochloride [pH 7.9], 1.5 mM $MgCl_2$, 0.65% Nonidet P-40) and agitated gently on ice until lysis; the nuclei were removed by centrifugation. The cytoplasmic supernatant was adjusted to 0.5% SDS and 1 mM EDTA, extracted twice with phenol-chloroform (1:1, saturated with 0.01 M sodium acetate [pH 5.5], 0.1 M NaCl, 1 mM EDTA), followed by two extractions with chloroform-isoamyl alcohol (24:1). The final aqueous phase was made 0.2 M sodium acetate (pH 5.5), and 2.5 volumes of ethanol were then added. The ethanol precipitate was pelleted, washed two times with 70% ethanol dried under vacuum, dissolved in water, and stored at −20°C. To obtain DNA-free RNA, a modification of a CsCl purification method was used (11); this involved preparing cytoplasmic RNA by the Nonidet P-40–phenol–chloroform procedure and then centrifuging the RNA through a 5.7 M CsCl–50 mM EDTA cushion. Poly(A)$^+$ RNA was prepared by affinity to oligodeoxythymidylic acid-cellulose (2) purchased from Collaborative Research, Inc., Waltham, Mass.

Cell-free translation. RNA was translated in the reticulocyte lysate system of Pelham and Jackson (34), purchased from New England Nuclear.

mRNA-DNA hybrid selection and cell-free translation. Plasmid DNA was covalently attached to identical diazobenzyloxymethyl filters (approximately 4 by 4 mm) by the method of Alwine et al. (1); each filter contained 7 μg of β-actin cDNA plasmid (5 μg of pBR322 sequences and 2 μg of β-actin cDNA sequences). Hybridization conditions were those of Barnett et al. (3): 37°C overnight in 50% formamide–0.6 M NaCl–80 mM Tris-hydrochloride (pH 7.8)–4 mM EDTA–0.1% SDS. After hybridization the filters were washed 10 times at 37°C in 1 ml of solution containing 50% formamide–30 mM NaCl–4 mM Tris-hydrochloride (pH 7.8)–0.2 mM EDTA–0.1% SDS. The specifically hybridized mRNA was eluted from the filters by incubation at 65°C for 3 to 5 min in 99% formamide–20 mM Tris-hydrochloride (pH 7.8); calf liver tRNA (Boehringer Mannheim Corp., New York, N.Y.) was added to eluted mRNA as carrier, and samples were concentrated by ethanol precipitation.

[^{3}H]poly(U) synthesis and assays. ^{3}H-labeled polyuridylic acid [poly(U)] was synthesized by a modification

of the procedure of Bishop et al. (6). Polynucleotide phosphorylase was purchased from Miles Laboratories, Inc., Elkhart, Ind.; [^{3}H]UDP (12.7 Ci/mmol) was from Amersham Corp., Arlington Heights, Ill.; unlabeled UDP was from P-L Biochemicals Inc., Milwaukee, Wis. Reaction conditions were as follows: 10 mM Tris-hydrochloride (pH 9.0), 5 mM $MgCl_2$, 0.4 mM EDTA, 0.2 mg of bovine serum albumin per ml, 200 U of polynucleotide phosphorylase per ml, 5 mCi of [^{3}H]UDP per ml, and 9.2 mg of unlabeled UDP per ml. The reaction was incubated at 37°C for 30 min; this gave approximately 30% incorporation of label. The reaction was phenol extracted, followed by ether extraction, and run over a Sephadex G-100 column in 5 mM Tris-hydrochloride (pH 7.9)–5 mM EDTA–10 mM NaCl. Fractions were collected, and portions were spotted on GF/C glass fiber filters (Whatman) and CTAB (cetyl [C16] trimethylammonium bromide; Sigma Chemical Co., St. Louis, Mo.) precipitated to determine percent precipitable counts per minute in each fraction. Fractions with greater than 30% CTAB precipitability were pooled, optical density at 260 nm was measured to determine concentration, and samples were stored at −70°C. Two [^{3}H]poly(U) preparations synthesized under these conditions had specific activities of 150 cpm/ng and 350 cpm/ng.

Hybridization conditions were routinely in a 100 μl volume in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 42°C for 1 h with a 10-fold excess of [^{3}H]poly(U) to poly(A) content. After hybridization, the reaction was cooled on ice for 5 min and digested with pancreatic RNase at 100 μg/ml at room temperature for 45 min. Samples were CTAB precipitated by the addition of 2 volumes of 0.1% yeast tRNA (Sigma; in 2 N sodium acetate, pH 5.5), followed by 3 volumes of 3% CTAB. The precipitates were filtered over GF/C glass fiber filters, washed with 10 ml of cold water, dried, and counted in nonaqueous liquid scintillation fluid.

Northern blots. The procedure used for formaldehyde-agarose Northern blots was a modification of that of Seed and Goldberg (unpublished data). A 1.0% agarose gel was prepared by melting agarose in 1.2× gel buffer (1× gel buffer is 20 mM morpholinepropanesulfonic acid, 50 mM sodium acetate, 1 mM EDTA, pH 7.0). The agarose solution was cooled to 65°C, and then 1/6 volume of 37% formaldehyde (Mallinckrodt Inc., St. Louis, Mo.) was added to give a final concentration of 6% formaldehyde. The gel running buffer was 1× gel buffer without formaldehyde. RNA samples were incubated at 55°C for 15 min in 50% formamide–6% formaldehyde–1× gel buffer containing bromophenol blue.

After electrophoresis, the gel was incubated for 45 min at room temperature in 50 mM NaOH–100 mM NaCl; this partial alkaline hydrolysis improves the transfer of high-molecular-weight RNA. The gel was neutralized in two washes for 30 min in 0.1 M Tris-hydrochloride (pH 7.5) and then equilibrated in 20× SSC (two washes for 30 min). The RNA was transferred for 2 h to overnight in 20× SSC by the Southern procedure. After transfer the filter was washed in 3× SSC, air dried, and baked for 2 h in a 75°C vacuum oven.

DNA samples were ^{32}P labeled by nick translation by the method of Maniatis et al. (30). The specific activity of nick-translated probes was routinely 10^8 cpm/μg. Blots were prehybridized for at least 2 h and hybridized with the probe for 4 h to overnight as described by Alwine et al. (1). The washed blots were placed under preflashed X-ray film (Eastman Kodak Co., Rochester, N.Y.) with an intensifying screen and exposed at −70°C.

RESULTS

Rapid alterations in polypeptide synthesis during the infection of L cells with vaccinia virus. Confluent monolayers of L cells were infected at a multiplicity of 30 PFU per cell. Protein synthesis was monitored by labeling polypeptides with [^{35}S]methionine every hour throughout infection. The synthesis of polypeptides in uninfected cells was determined by subjecting plates of uninfected cells to the same changes of media and physical manipulations as infected plates and labeling with [^{35}S]methionine.

Cytoplasmic extracts were prepared by lysing the cells with Nonidet P-40. Equal volumes were fractionated in an SDS-polyacrylamide gel, and the [^{35}S]methionine-labeled polypeptides were visualized by fluorography (Fig. 1).

There is a progressive decline in protein synthesis obvious between 1 and 10 h of infection (Fig. 1). This was confirmed by measuring the incorporation of [^{35}S]methionine into trichloroacetic acid-precipitable polypeptides throughout infection. In fact, after 10 h of infection with vaccinia virus total L-cell protein synthesis was reduced to 44% of that of mock-infected cells.

Infection also induces a rapid decline in the synthesis of cellular polypeptides so that by 6 h of infection their synthesis is totally shut off (Fig. 1). This is most dramatically exemplified in the cessation of synthesis of actin, a major [^{35}S]methionine-labeled polypeptide in uninfected L cells (Fig. 1). Two other, clearly resolved polypeptides, denoted 80K and 22K, are also obvious examples of this virus-induced shutoff of L-cell protein synthesis.

Concurrent with the inhibition of L-cell protein synthesis there is an orderly temporal expression of vaccinia virus polypeptides. These polypeptides were originally classified by Salzman and Sebring (40) according to whether they were synthesized before or after viral DNA replication. In these experiments presented here, viral DNA synthesis initiates at 1.5 h p.i, peaks at 3 to 5 h, and is complete after 5 h (R. Jones and A. Rice, unpublished data). That class synthesized before DNA replication are called "early" polypeptides, and their synthesis is initiated within the first hour of infection and terminated approximately at 5 h along with DNA replication. Two examples of early polypeptides are those denoted 28K and 24K in Fig. 1. In contrast, "late" polypeptides constitute a class of viral genes whose synthesis is dependent upon viral DNA replication. Two examples of

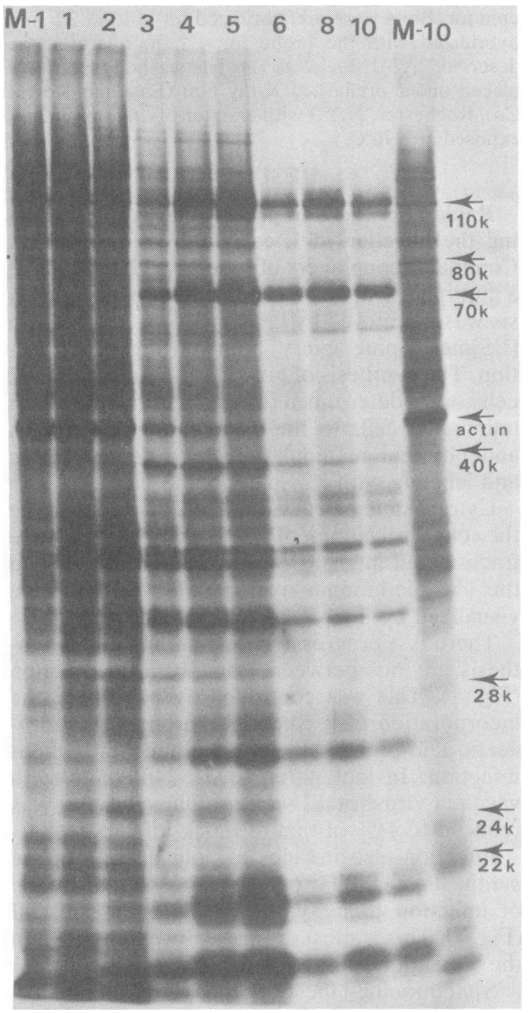

FIG. 1. Time course of polypeptide synthesis during vaccinia virus infection in L cells. L-cell culture dishes (35 mm) were infected with vaccinia virus at 30 PFU per cell. At intervals after infection, from 1 to 10 h, a dish of infected cells was pulse-labeled with [^{35}S]methionine for 15 min. The times of labeling are noted at the top of the figure from 1 to 10 h after infection. Two dishes of uninfected cells served as mock-infected controls. One dish was labeled at 1 h after infection (M-1), and the other dish was labeled at 10 h after infection (M-10). Equal portions of cell lysates were loaded in each lane. Electrophoresis was in a 10% gel. The gel was processed for fluorography.

late polypeptides are denoted 110K and 70K in Fig. 1. Their synthesis is initiated after 3 h and continues unabated throughout the infectious cycle. Another class of viral polypeptides called "constitutive" polypeptides were originally identified by Pennington (35). Their synthesis is initiated within the first hour of infection, before DNA synthesis, and continues unaltered throughout the infection. An obvious example of a polypeptide belonging to this constitutive class is denoted 40K in Fig. 1.

Alterations in the translatable cellular and viral mRNA population during vaccinia virus infection. The rapid changes in protein synthesis during vaccinia virus infection in intact cells (Fig. 1) could reflect either changes in the mRNA population or profound alterations of the protein synthetic apparatus. To distinguish between these possibilities we have isolated RNA from uninfected and infected cells and translated it in both heterologous and homologous cell-free systems.

Total cytoplasmic RNAs from uninfected L cells and cells at 1 and 7 h p.i. were fractionated into poly(A)$^+$ and poly (A)$^-$ populations by oligodeoxythymidylic acid-cellulose chromatography. These RNA populations were translated in the message-dependent reticulocyte lysate (34), and the [^{35}S]methionine-labeled polypeptides were analyzed on an SDS-polyacrylamide gel (Fig. 2). It is clear the majority of L-cell mRNA and vaccinia virus mRNA is poly(A)$^+$, with the obvious exceptions of two cellular mRNAs, that encoding a 14K polypeptide and a portion of actin mRNA. Moreover, the dramatic changes in protein synthesis obvious in intact cells are mirrored in the pattern of polypeptides directed by the isolated mRNAs. Minor exceptions result from post-translational modifications of particular polypeptides. These results indicate that switches in protein synthesis during vaccinia virus infection result directly from changes in translatable mRNA levels, rather than from alterations in the protein synthetic apparatus, in agreement with the study of Cooper and Moss (11) in vaccinia virus-infected HeLa cells.

This conclusion was supported by utilizing homologous cell-free systems prepared from uninfected and vaccinia virus-infected L cells. Both systems were capable of translating L cell, adenovirus, and vaccinia virus mRNAs. Moreover, in each case the pattern of polypeptides directed by both extracts was identical, indicating no inability of the infected extract to translate cellular or viral mRNA (data not shown). This contrasts with results in homologous cell-free systems prepared from poliovirus-infected HeLa cells (39, 46) and reovirus-infected L cells (43), where there is a dramatic preference for the translation of uncapped viral mRNAs over 5′ capped cellular mRNAs.

Similar to the shutoff of cellular polypeptide synthesis in cells, cellular polypeptides directed by cell-free translation of isolated RNA diminish rapidly during infection. This is most dramatically exemplified by actin, a major translation product in all L-cell RNA populations shown in Fig. 2, which is not detectable by cell-free translation after 7 h of infection. Another exam-

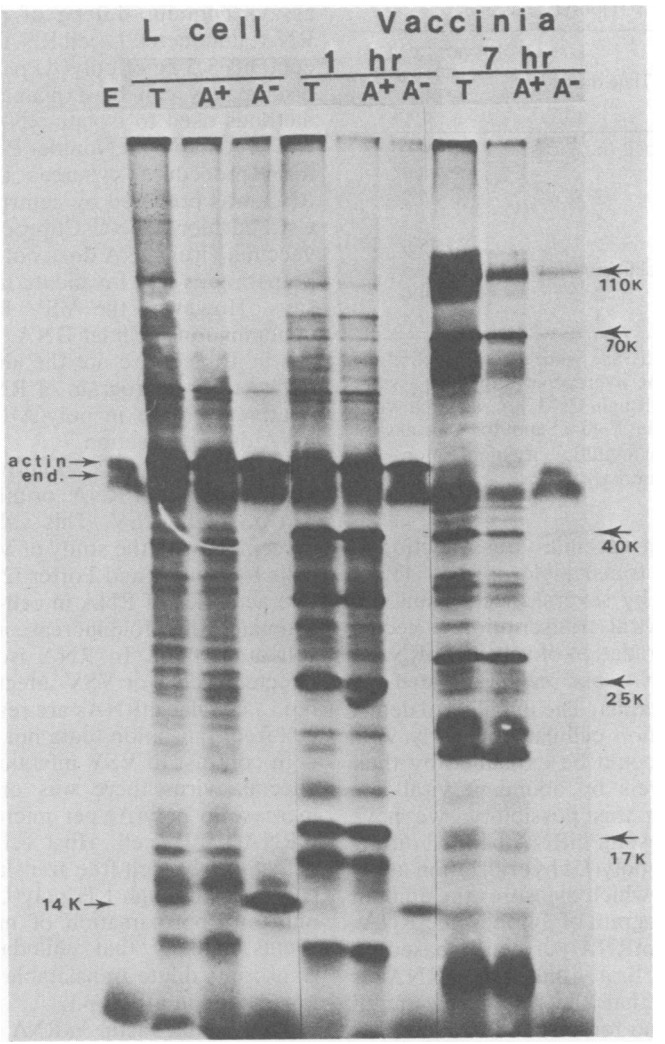

FIG. 2. Cell-free translation products of RNA extracted from uninfected L cells and vaccinia virus-infected L cells at 1 and 7 h after infection. L-cell culture dishes (150 mm) were infected with vaccinia virus at 30 PFU per cell. Infected cells were harvested at 1 and 7 h after infection, and uninfected cells were harvested at 7 h. Total cytoplasmic RNA was prepared from each batch of cells and fractionated into poly(A)$^+$ and poly(A)$^-$ RNA by chromatography on oligodeoxythymidylic acid-cellulose. The RNA preparations were translated in the reticulocyte lysate system at the following concentrations: total cytoplasmic RNA (T), 5 μg per 25-μl reaction; poly(A)$^+$ RNA (A$^+$), approximately 1 μg per 25-μl reaction; poly(A)$^-$ RNA (A$^-$), 5 μg per 25-μl reaction; endogenous reaction (E), no added RNA. From 100,000 to 150,000 cpm was loaded in each lane, except lane E. The polypeptide denoted end indicates an endogenous [^{35}S]methionine polypeptide of the reticulocyte lysate; in this gel this endogenous polypeptide has migrated slightly below actin. Electrophoresis was in a 17.0% gel. The gel was processed for fluorography.

ple is the 14K polypeptide present in L cell poly(A)$^-$ RNA; it is absent in translations of poly(A)$^-$ RNA at 7 h p.i.

Coincident with the attenuation and ultimate shutoff of cellular polypeptides, the virus is accomplishing an orderly temporal expression of its genetic information. Translatable viral mRNAs encoding the classes of polypeptides defined above as early, constitutive, and late are observable in Fig. 2. Two examples of early mRNAs are those coding polypeptides denoted 25K and 17K, which are present at high levels at 1 h p.i. and, along with cellular mRNAs, disappear at 7 h. In contrast, a viral mRNA encoding a constitutive 40K polypeptide is present at high levels at both 1 and 7 h p.i. Examples of late mRNAs are those encoding 110K and 70K polypeptides, present only at 7 h p.i. These drastic

TABLE 1. [^{3}H]poly(U) assays[a]

Virus	Time (h) p.i.	ng of poly(A) per μg of cytoplasmic RNA
	L cell (no virus)	4.0
Vaccinia virus	1	4.3
Vaccinia virus	3	5.2
Vaccinia virus	5	5.5
Vaccinia virus	7	5.9
	L cell (no virus)	3.5
VSV	2	4.0
VSV	4	9.4

[a] The conditions of assays were as described in the text. Each value is the average of duplicate assays. RNA was purified through CsCl for vaccinia virus assays and with Nonidet P-40–phenol for VSV assays. Infections were done at a multiplicity of infection of 30 in L cells grown as monolayers.

changes seen during vaccinia virus infection in cellular and viral translatable mRNA levels could be explained by several mechanisms, including abundant viral transcription, selected inactivation, or degradation of specific mRNAs.

Amount of mRNA per cell estimated by [^{3}H]poly(U) hybridization. The inability to detect by cell-free translation cellular and early viral mRNA at 7 h p.i. could be explained by their dilution to low levels by abundant viral late transcripts. To test this possibility, we have estimated the increase in mRNA per cell during infection by the [^{3}H]poly(U) hybridization assay of Bishop et al. (6), which measures the amount of poly(A) per microgram of cytoplasmic RNA.

This measure of mRNA per cell is based on two assumptions: first, that all mRNA is poly(A)$^+$; second, that the length of the 3′ poly(A) tract is similar for host and viral mRNA. The first assumption is substantiated, as chromatography on oligodeoxythymidylic acid-cellulose demonstrates that the majority of in vitro-translatable mRNA is poly(A)$^+$ in uninfected and vaccinia virus-infected L cells (Fig. 2). Nevins and Joklik (32) have shown that the poly(A) tract is similar in size in L cells and vaccinia virus-infected cells, the median length being 100 bases, thus validating the second assumption.

For comparison we have used [^{3}H]poly(U) hybridization to estimate the increase in mRNA per cell during VSV infection in L cells. VSV is a cytoplasmic RNA virus and has been shown to inhibit cellular protein synthesis by simply transcribing abundant viral mRNA that dilutes and competes with cellular mRNA for limited ribosomes (27). The assumptions used for the [^{3}H]poly(U) assay as a measure of mRNA per cell are also valid for VSV-infected cells (13, 38).

Uninfected L-cell RNA in the vaccinia virus assays contains 4.0 ng of poly(A) per μg of RNA; uninfected L-cell RNA in the VSV assays contains 3.5 ng of poly(A) per μg of RNA. This discrepancy can be explained by the different methods used to isolate RNA. The VSV RNA was prepared by a Nonidet P-40–phenol–chloroform procedure, whereas the vaccinia virus RNA was prepared by centrifugation through a CsCl cushion. The CsCl procedure ensures that vaccinia virus DNA does not contaminate RNA preparations and invalidate the [^{3}H]poly(U) assays. However, the VSV RNA may contain contaminating cellular DNA. The important values in Table 1 are not the absolute amounts of poly(A) per microgram of RNA, but rather the relative increase in poly(A) per microgram of RNA during infection.

There was a net 2.7-fold increase in poly(A) per microgram of RNA, or mRNA per L cell, at 4 h p.i. with VSV. This value is in excellent agreement with the study of VSV-infected BHK cells by Lodish and Porter (27), who used relative activities of RNA in cell-free translation to estimate a threefold increase in mRNA per BHK cell at 4 h p.i. In RNA isolated from VSV-infected L cells or VSV-infected BHK cells at 4 h p.i., cellular mRNAs are readily detectable by cell-free translation (data not shown; 27, 28).

In contrast to VSV infection, at 7 h p.i. with vaccinia virus there was only a net 1.4-fold increase in poly(A) per microgram of RNA or mRNA per L cell. Host cell mRNAs are not detectable by cell-free translation of this RNA (Fig. 2). Although [^{3}H]poly(U) hybridization is only an approximation of mRNA per cell, it seems unlikely that abundant vaccinia virus transcripts dilute translatable L-cell mRNA below detection at 7 h p.i.

Translatable actin mRNA is absent late in vaccinia virus infection. Alternatively, vaccinia virus transcripts may impede the translation of cellular mRNAs. To test this a specific cellular mRNA was selected by hybridization to its complementary DNA, and the enriched mRNA was translated in the reticulocyte lysate. For this purpose we have taken advantage of the evolutionary conservation at the nucleotide level of the actin gene and used a previously characterized chicken β-actin cDNA plasmid (9) to select this abundant L-cell mRNA. Cleveland et al. (9) have shown that the β-actin plasmid shares homology with α- and β-actin mRNAs of chickens; it is reasonable to assume that the plasmid also shares homology with mouse L cell α- and β-actin mRNAs. We refer to the L cell mRNA(s) with which the chicken β-actin cDNA plasmid hybridizes as simply actin mRNA.

Seven identical filters were prepared by covalently attaching 7 μg of β-actin plasmid to diazobenzyloxymethyl paper. A series of dilutions of

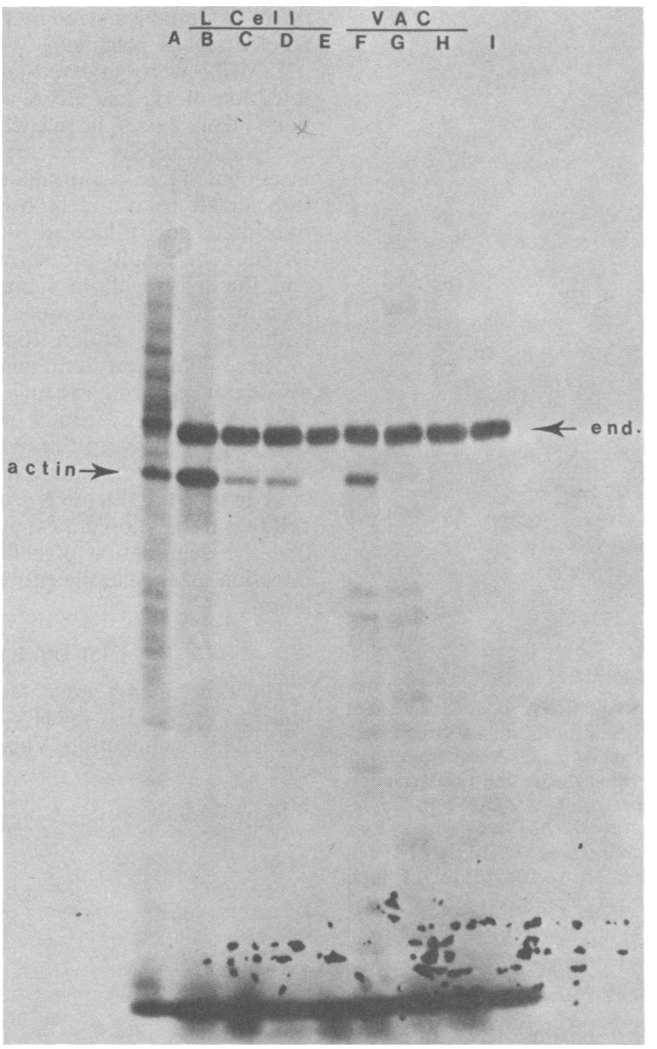

FIG. 3. Cell-free translation of actin-selected mRNA. A β-actin cDNA plasmid was covalently attached to diazobenzyloxymethyl paper. Each filter contained 7 μg of plasmid DNA (5 μg of pBR322 vector sequences and 2 μg of β-actin cDNA sequences). Lane A contains the translation products of total cytoplasmic RNA isolated from uninfected L cells. The lanes denoted L Cell contain the translation products of actin mRNA selected from (B) 100 μg of L-cell cytoplasmic RNA, (C) 50 μg of L-cell cytoplasmic RNA plus 50 μg of calf liver tRNA, (D) 20 μg of L-cell cytoplasmic RNA plus 80 μg of tRNA, and (E) 2 μg of L-cell cytoplasmic RNA plus 98 μg of tRNA. The lanes denoted VAC contain the translation products of actin mRNA selected from 100 μg of total cytoplasmic RNA isolated from vaccinia-infected L cells at (F) 2 h after infection, (G) 4 h after infection, and (H) 8 h after infection. Lane I contains the translation products of an endogenous reticulocyte lysate reaction, that is, no added RNA. In this gel the endogenous [^{35}S]methionine-labeled polypeptide (end.) of the reticulocyte lysate migrated above actin. Electrophoresis was in a 10% gel. The gel was processed for fluorography.

L-cell total cytoplasmic RNA (ranging from 100 to 2 μg) was hybridized to four filters. Each hybridization was supplemented with calf liver tRNA to bring the RNA mass to 100 μg per filter. The three remaining filters were hybridized with 100 μg of cytoplasmic RNA from vaccinia virus-infected cells at 2, 4, and 8 h p.i. After hybridization the filters were extensively washed to remove nonspecifically bound RNA; the bound actin mRNA was then eluted from the filters and translated in the reticulocyte lysate, and the products are shown in Fig. 3.

Lanes B, C, D, and E in Fig. 3 contain the translation products of actin mRNA selected from 100, 50, 10, and 2 μg of total cytoplasmic RNA, respectively. This demonstrates the limit of detection of this particular mRNA selection and shows that enough translatable mRNA is

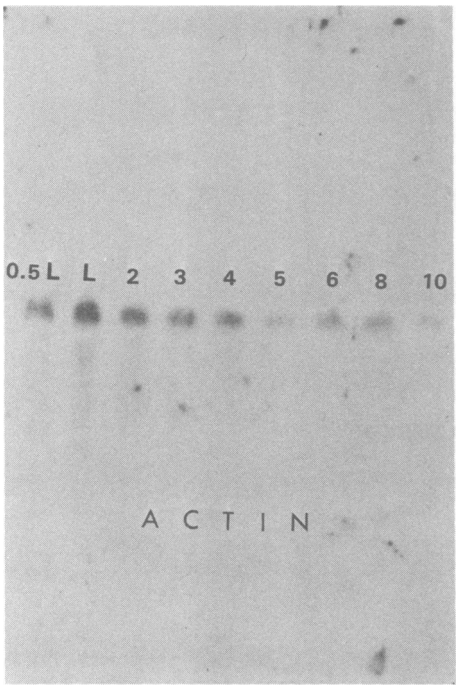

FIG. 4. Analysis of actin mRNA sequences present in total cytoplasmic RNA during vaccinia virus infection in L cells. Total cytoplasmic RNA was fractionated in a 1.5% formaldehyde–agarose gel, transferred to nitrocellulose filter paper, and hybridized to ^{32}P-labeled, nick-translated actin cDNA. Lanes: (0.5 L) 5 μg of L-cell total cytoplasmic RNA; (L) 10 μg of L-cell total cytoplasmic RNA; (2 to 10) 10 μg of total cytoplasmic RNA from vaccinia-infected L cells at 2 to 10 h p.i., respectively. L cells were infected with 15 PFU per cell.

present in 10 μg of cytoplasmic RNA to give a detectable product (lane D). Therefore, this experiment readily assays a 10-fold reduction in translatable actin mRNA (compare lanes B and D).

Lanes F, G, and H in Fig. 3 contain the translation product of actin mRNA selected from 100 μg of RNA isolated at 2, 4, and 8 h p.i. It is clear that translatable actin mRNA disappears late in vaccinia virus infection.

Actin and α-tubulin mRNA sequences are degraded during vaccinia virus infection. The absence of translatable actin mRNA late in infection indicates either an inactivation or degradation of cellular mRNAs. To distinguish between these possibilities the presence of cellular mRNA sequences during infection was assayed by hybridization with two highly conserved sequences, chicken β-actin (9) and rat α-tubulin (25) cDNA clones. RNA was fractionated according to size in a denaturing gel system and transferred to filter paper, and individual mRNAs were visualized by hybridization to ^{32}P-labeled cDNA plasmids.

L cells were infected at a multiplicity of infection of 15, and RNA was isolated at time points from 2 to 10 h. In each case 10 μg of total cytoplasmic RNA was loaded in each lane, fractionated in a formaldehyde-agarose gel, transferred to nitrocellulose filter paper, and hybridized to ^{32}P-labeled β-actin and α-tubulin DNAs. The results are shown in Fig. 4 and 5, and the hybridization signals of actin and α-tubulin mRNAs were quantitated by scanning with a Joyce-Lobel densitometer (Fig. 6).

These data show actin and α-tubulin mRNAs are degraded during vaccinia virus infection in L cells. Moreover, cellular mRNA degradation occurs progressively throughout vaccinia virus infection. No intermediate breakdown products of actin or α-tubulin mRNA are observable. The cell-free translation data shown in Fig. 2 suggest that the degradation of cellular mRNA during infection extends to the entire L-cell RNA population.

DISCUSSION

The experiments presented in Fig. 2, 4, and 6 indicate that L-cell mRNA is degraded during vaccinia virus infection. Vaccinia virus infection

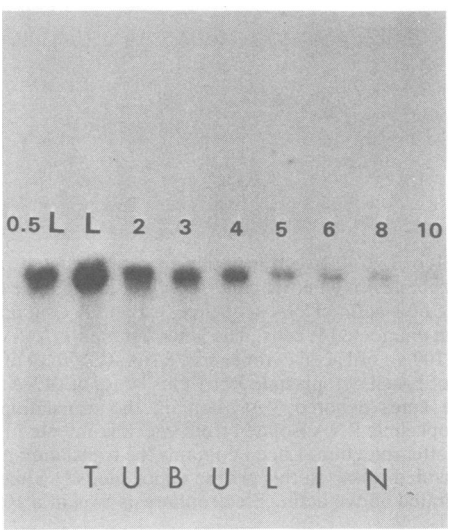

FIG. 5. Analysis of α-tubulin mRNA sequences present in total cytoplasmic RNA during vaccinia virus infection in L cells. Total cytoplasmic RNA was fractionated in a 1% formaldehyde–agarose gel, transferred to nitrocellulose filter paper, and hybridized to ^{32}P-labeled, nick-translated α-tubulin cDNA. Lanes: (0.5 L) 5 μg of L-cell total cytoplasmic RNA; (L) 10 μg of L-cell total cytoplasmic RNA; (2 to 10) 10 μg of total cytoplasmic RNA from vaccinia virus-infected L cells at 2 to 10 h p.i., respectively. L cells were infected with 15 PFU per cell.

is known to induce rapid inhibition of cellular RNA synthesis; in HeLa cells both nuclear rRNA and mRNA synthesis were reported to be switched off after 3 h of infection (4), whereas in L cells little or no rRNA synthesis was seen after 3 h (20). It is possible the conditions of infection used in the experiments presented here induce a more rapid inhibition of cellular transcription, and the degradation of cellular mRNA seen could simply represent the normal turnover of mRNA species after their synthesis was blocked. Alternatively, if L-cell mRNAs possess long half-lives in uninfected cells, then infection would actually induce an accelerated degradation of cellular mRNA. To distinguish between these possibilities it is necessary to know the half-lives of L cell mRNAs in uninfected cells.

Previous determinations of mRNA turnover in L cells and HeLa cells indicate that the half-life of the poly(A)$^+$ mRNA population in cultured mammalian cells correlates with the cell generation time, ranging from 20 to 40 h depending on growth conditions (14, 36, 42). The L cells used in the experiments presented here have a doubling time of approximately 20 h. However, a recent study using cloned genes in CHO cells indicates that a substantial fraction of mRNAs may have lifetimes as short as 3 h (17).

We have estimated the half-lives of L-cell actin and α-tubulin mRNAs to both be greater than 6 h in growing L cells (A. P. Rice, Ph.D. thesis, Brandeis University, Waltham, Mass., 1982) by the following two techniques: (i) actinomycin D was used to block transcription, and the decay of actin and tubulin mRNAs was measured by Northern blotting techniques; (ii) L cell RNA was labeled in vivo for 8 h with ^{32}P, and the kinetics of incorporation of label into actin and α-tubulin mRNAs was measured by the technique of Mangriotti et al. (29). By 3 h in vaccinia virus-infected L cells actin and α-tubulin mRNAs are degraded to a level 50% or less of that of uninfected cells (Fig. 6); if actin and α-tubulin mRNAs have normal half-lives greater than 6 h, then this indicates that vaccinia virus infection causes accelerated degradation of cellular mRNA.

What brings about this rapid degradation of cellular mRNA during vaccinia virus infection? It could be achieved by a number of mechanisms: the activation of a latent cellular RNase, the synthesis of a cellular or viral nuclease, the release of a viral encapsulated nuclease, or recompartmentalization of mRNA within the infected cell, rendering it more susceptible to enhanced turnover. There exist precedents for these possibilities. In interferon-treated cells a series of 2′-5′-linked oligoadenylic acid triphosphates are synthesized; 2′-5′-linked oligoadenylic acid triphosphate activates an endogenous

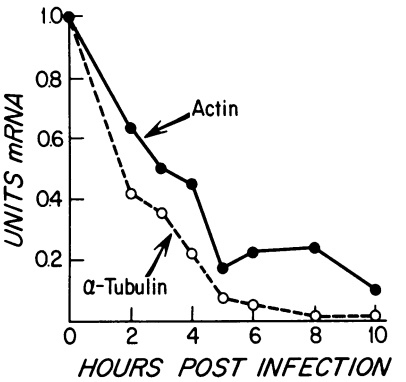

FIG. 6. Quantitation of autoradiographs shown in Fig. 4 and 5. The autoradiographs were quantitated by scanning with a Joyce-Lobel densitometer. An arbitrary value of 1.0 U of actin mRNA and α-tubulin mRNA was assigned to the bands in lanes L (10 μg of L-cell total cytoplasmic RNA).

nuclease that is capable of degrading both viral and cellular mRNA (47). The vaccinia virion is known to contain two single strand-specific DNases localized in the viral core. It remains to be seen whether one or both of these nucleases, or a different nuclease contained in the virion, possess RNase activity (37). Vaccinia virus infection is also known to induce rapid morphological changes in L cells (16). These morphological changes could cause recompartmentalization of mRNAs within the cell which might be involved in accelerated mRNA turnover. Viral infection can indeed cause relocalization of mRNA within the infected cell, as poliovirus has been shown to shuttle cellular mRNA from attachment to the cytoskeleton to the soluble cytoplasmic component of infected HeLa cells (26). However, this relocalization of cellular mRNA during poliovirus infection is not sufficient by itself to enhance mRNA turnover, as cellular mRNA is known to remain stable during poliovirus infection (24). In summary, one or more of these possibilities may explain the mechanism of accelerated degradation of cellular mRNA during vaccinia virus infection; vaccinia virus presents itself as an attractive model system for future studies of mRNA turnover in eucaryotic cells.

This finding that cellular mRNA undergoes rapid degradation during infection has profound implications as to how vaccinia virus regulates the temporal expression of its own genetic information. Before viral DNA replication, the expression of two classes of viral polypeptides is initiated: "early" polypeptides whose synthesis is switched off coincident with those of the cell, and "consitutive" polypeptides whose synthesis continues throughout infection. Coincident with the onset of DNA replication the synthesis of "late" polypeptides is initiated. The vaccinia

virus infectious cycle concludes with the assembly of virion particles in the cell cytoplasm. A future understanding of how vaccinia virus regulates levels of viral mRNA during infection must consider the ongoing rapid turnover of cellular mRNA. It is remarkable that the ordered regulation of expression of the vaccinia virus genome is able to proceed in the midst of this extensive degradation of mRNA.

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ADDENDUM IN PROOF

It has recently been shown that cellular mRNA is degraded during influenza virus and herpes simplex virus infections (S. C. Inglis, Mol. Cell. Biol. **2**:1644–1648, 1982).

LITERATURE CITED

1. **Alwine, J. C., D. T. Kemp, B. A. Parker, J. Reiser, J. Renant, G. R. Stark, and G. M. Wahl.** 1980. Detection of specific RNAs on specific fragments of DNA by fractionation in gels and transfer to diazobenzyloxymethyl paper. Methods Enzymol. **68**:220–242.
2. **Aviv, H., and P. Leder.** 1972. Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. Proc. Natl. Acad. Sci. U.S.A. **69**:1408–1412.
3. **Barnett, T., C. Pachl, J. P. Gergen, and P. Wensink.** 1980. The isolation and characterization of *Drosophila* yolk protein genes. Cell **21**:729–738.
4. **Becker, Y., and W. K Joklik.** 1964. Messenger RNA in cells infected with vaccinia virus. Proc. Natl. Acad. Sci. U.S.A. **51**:577–585.
5. **Beltz, G. A., and S. J. Flint.** 1979. Inhibition of HeLa cell protein synthesis during adenovirus infection. J. Mol. Biol. **131**:353–373.
6. **Bishop, J. O., M. Rosbash, and D. Evans.** 1974. Polynucleotide sequences in eukaryotic DNA and RNA that form ribonuclease-resistant complexes with polyuridylic acid. J. Mol. Biol. **85**:75–86.
7. **Bonner, W. M., and R. A. Laskey.** 1974. A film detection method for tritium-labelled proteins and nucleic acids in polyacrylamide gels. Eur. J. Biochem. **46**:83–88.
8. **Boone, R. F., and B. Moss.** 1978. Sequence complexity and relative abundance of vaccinia virus mRNA's synthesized in vivo and in vitro. J. Virol. **26**:554–569.
9. **Cleveland, D. W., M. A. Lopata, R. J. MacDonald, N. J. Cowan, W. J. Rutter, and M. W. Kirschner.** 1980. Number and evolutionary conservation of α- and β-tubulin and cytoplasmic α- and β-actin genes using specific cloned cDNA probes. Cell **20**:95–105.
10. **Cooper, J. A., and B. Moss.** 1978. Transcription of vaccinia virus mRNA coupled to translation *in vitro*. Virology **88**:149–165.
11. **Cooper, J. A., and B. Moss.** 1979. *In vitro* translation of immediate early, early and late classes of mRNA from vaccinia-virus infected cells. Virology **96**:368–380.
12. **Crawford, N., A. Fire, M. Samuels, P. G. Sharp, and D. Baltimore.** 1981. Inhibition of transcription factor activity by poliovirus. Cell **27**:555–561.
13. **Ehrenfeld, E., and D. Summers.** 1972. Adenylate-rich sequences in vesicular stomatitis virus messenger ribonucleic acid. J. Virol. **10**:683–688.
14. **Greenberg, D. R.** 1972: High stability of messenger RNA in growing cultured cells. Nature (London) **240**:102–104.
16. **Hanafusa, H.** 1960. Killing of L cells by heat-and-UV-inactivated vaccinia virus. Biken J. **3**:191–199.
17. **Harpold, M. M., M. C. Wilson, and J. E. Darnell.** 1981. Chinese hamster polyadenylated messenger ribonucleic acid: relationship to non-polyadenylated sequences and relative conservation during messenger ribonucleic acid processing. Mol. Cell. Biol. **1**:188–198.
18. **Herz, C., E. Stavner, R. M. Krug, and T. Gurney, Jr.** 1981. Influenza virus, an RNA virus, synthesizes its messenger RNA in the nucleus of infected cells. Cell **26**:391–400.
19. **Hruby, D. E., L. A. Guarino, and J. R. Kates.** 1979. Vaccinia virus replication. I. Requirement for the host-cell nucleus. J. Virol. **29**:705–715.
20. **Jefferts, E. R., and J. A. Holowczak.** 1971. RNA synthesis in vaccinia-infected L cells: inhibition of ribosome formation and maturation. Virology **46**:730–744.
21. **Kaufmann, Y., E. Goldstein, and S. Penman.** 1976. Poliovirus-induced inhibition of polypeptide initiation *in vitro* on native polyribosomes. Proc. Natl. Acad. Sci. U.S.A. **73**:1834–1838.
22. **Kit, S., and D. R. Dubbs.** 1962. Biochemistry of vaccinia-infected mouse fibroblasts (strain L-M). Virology **18**:274–285.
23. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
24. **Leibowitz, R., and S. Penman.** 1971. Regulation of protein synthesis in HeLa cells. III. Inhibition during poliovirus infection. J. Virol. **8**:661–668.
25. **Lemishka, J. R., S. Farmer, V. R. Racaniello, and P. A. Sharp.** 1981. Nucleotide sequence and evolution of a mammalian α-tubulin messenger RNA. J. Mol. Biol. **151**:101–120.
26. **Lenk, R., and S. Penman.** 1979. The cytoskeletal framework and poliovirus metabolism. Cell **16**:289–301.
27. **Lodish, H. F., and M. Porter.** 1980. Translational control of protein synthesis after infection by vesicular stomatitis virus. J. Virol. **36**:719–733.
28. **Lodish, H. F., and M. Porter.** 1981. Vesicular stomatitis virus mRNA and inhibition of translation of cellular mRNA—is there a P function in vesicular stomatitis virus? J. Virol. **38**:504–517.
29. **Mangriotti, G., P. Lefebvre, and H. F. Lodish.** 1982. Differences in the stability of developmentally regulated mRNAs in aggregated and disaggregated *Dictyostelium discordeum* cells. Dev. Biol. **89**:82–91.
30. **Maniatis, T., A. Jeffrey, and D. G. Kleid.** 1975. Nucleotide sequence of the rightward operator of phage. Proc. Natl. Acad. Sci. U.S.A. **72**:1184–1188.
31. **Moss, B.** 1979. *In* D. P. Nayak (ed.), The molecular biology of animal viruses. vol. 2, p. 849–890. Marcel Dekker, New York.
32. **Nevins, J. R., and W. K. Joklik.** 1975. Poly (A) sequences of vaccinia virus messenger RNA: nature, mode of addition and function during transcription *in vitro* and *in vivo*. Virology **63**:1–14.
33. **Nishioka, Y., and S. Silverman.** 1977. Degradation of cellular mRNA during infection by herpes simplex virus. J. Virol. **27**:619–627.
34. **Pelham, H. R. B., and R. L. Jackson.** 1976. An efficient mRNA-dependent translation system from reticulocyte lysates. Eur. J. Biochem. **67**:247–256.
35. **Pennington, T. H.** 1974. Vaccinia virus polypeptide synthesis: sequential appearance and stability of pre- and post-replicative polypeptides. J. Gen. Virol. **25**:433–444.
36. **Perry, R. P., and D. E. Kelley.** 1973. Messenger RNA turnover in mouse L cells. J. Mol. Biol. **79**:681–696.
37. **Pogo, B. G. T., and S. Dales.** 1969. Two deoxyribonuclease activities within purified vaccinia virus. Proc. Natl. Acad. Sci. U.S.A. **63**:820–827.
38. **Rose, J. K., and D. Knipe.** 1975. Nucleotide sequence complexities, molecular weights, and polyadenylic acid content of the vesicular stomatitis virus mRNA species. J.

Virol. 15:994–1003.
39. **Rose, J. K., H. Traschel, K. Leong, and D. Baltimore.** 1978. Inhibition of translation by poliovirus: inactivation of a specific initiation factor. Proc. Natl. Acad. Sci. U.S.A. 75:2732–2736.
40. **Salzman, N. P., and E. D. Sebring.** 1967. Sequential formation of vaccinia virus protein and viral deoxyribonucleic acid replication. J. Virol. 1:16–23.
41. **Shatkin, A. J.** 1963. Actinomycin D and vaccinia virus infection of HeLa cells. Nature (London) 199:357–358.
42. **Singer, R. H., and S. Penman.** 1973. Messenger RNA in HeLa cells: kinetics of formation and decay. J. Mol. Biol. 78:321–334.
43. **Skup, D., H. Zarbl, and S. Millward.** 1981. Regulation of translation in L cells infected with reovirus. J. Mol. Biol. 151:35–55.
44. **Stenberg, R. M., and L. I. Pizer.** 1982. Herpes simplex virus-induced changes in cellular and adenovirus RNA metabolism in an adenovirus type 5-transformed human cell line. J. Virol. 42:474–487.
45. **Thomas, P. S.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. U.S.A. 77:5201–5205.
46. **Traschel, H., N. Sonenberg, A. J. Shatkin, J. K. Rose, K. Leong, J. E. Bergmann, J. Gordon, and D. Baltimore.** 1980. Purification of a factor that restores translation of vesicular stomatitis virus mRNA in extracts from poliovirus-infected HeLa cells. Proc. Natl. Acad. Sci. U.S.A. 77:770–774.
47. **Williams, B. R. G., R. R. Golgber, and J. M. Kerr.** 1979. Activation of a nuclease by ppp A2′p5′A2′p5′A in intact cells. FEBS Lett. 105:47–52.