JOURNAL OF VIROLOGY, Jan. 1988, p. 27–32
0022-538X/88/010027-06$02.00/0

Vol. 62, No. 1

La Crosse Virus Infection of Mammalian Cells Induces mRNA Instability

RAMASWAMY RAJU AND DANIEL KOLAKOFSKY*

Department of Microbiology, University of Geneva School of Medicine, CH-1211 Geneva 4, Switzerland

Received 24 June 1987/Accepted 16 September 1987

La Crosse virus infection of BHK cells leads to a dramatic shutoff of not only host protein synthesis but also viral protein synthesis later in infection. This shutoff can be accounted for by the loss of the cytoplasmic cellular and viral mRNAs. The induction of mRNA instability requires extensive virus replication, since when cycloheximide is added early in infection the preexisting viral and cellular mRNAs do not decrease upon incubation of the cultures. Pretreatment of the cultures with actinomycin D does not affect the ability of La Crosse virus infection to induce mRNA instability, and examination of the rRNAs shows no evidence of specific degradation due to activation of the interferon-associated latent RNase. The induction of mRNA instability therefore does not appear to operate through an interferon pathway. Viral mRNA synthesis, on the other hand, is not turned off during infection, and the cap-dependent endonuclease involved in viral mRNA initiation may be responsible for the mRNA instability.

La Crosse virus (LAC) is a member of the *Bunyavirus* genus of the *Bunyaviridae*, which are enveloped, trisegmented negative-strand RNA viruses. The genome segments are found both intracellularly and in virions as helical nucleocapsids and are designated small (S), medium (M), and large (L). These viruses grow in both insect and animal cells, but the fate of the infection is dramatically different depending on the species of the cell. The natural host for LAC is the mosquito *Aedes triseriatus*. In mosquitoes or in mosquito cells in culture the viral infection is asymptomatic, whereas in mammals or mammalian cells in culture the viral infection is associated with cytopathic effects (CPE). One possible reason for this difference in cytopathicity is that these viruses shut off host protein synthesis in mammalian cells but not in insect cells.

During the course of these studies on mammalian cells, we noticed that not only did LAC shut off host protein synthesis but also viral protein synthesis could be detected only relatively early in infection and then decayed with kinetics similar to that of the host protein synthesis. Further, virtually identical results have previously been reported for bunyamwera virus, another member of the *Bunyavirus* genus (12). The work reported here was undertaken to investigate the mechanism by which these bunyaviruses shut off viral as well as host protein synthesis in mammalian cells.

MATERIALS AND METHODS

Isolation of cytoplasmic RNAs. Confluent cultures of BHK-21, HeLa, HEL, or Vero cells in 10-cm-diameter dishes were infected with 20 to 50 PFU of LAC per cell. At the times indicated, the cells were harvested by scraping into phosphate-buffered saline and recovered by centrifugation. Cytoplasmic extracts were prepared by vortexing 10^7 cells in 0.2 ml of lysis buffer containing 0.15 M NaCl–10 mM Tris chloride (pH 7.4)–1 mM EDTA–0.5% Nonidet P-40 followed by centrifugation for 4 min at 4,000 × *g*. The extracts were then centrifuged on 20 to 40% preformed CsCl gradients for 16 h at 45,000 rpm in an SW60 rotor. The cytoplasmic RNA pellet thus obtained was dissolved in water and ethanol precipitated.

Northern and slot-blot analyses. Total cytoplasmic RNAs (20 μg) were heated for 2 min at 90°C in 80% formamide plus 0.1% xylene cyanol FF and electrophoresed on a 1.5-mm-thick 4% polyacrylamide gel containing 8 M urea. The gel was then soaked for 10 min in 10 mM Tris–5 mM sodium acetate–0.5 mM EDTA (pH 7.8) and electroblotted onto Hybond-N (Amersham Corp.) membrane in the same buffer. The membrane was then dried, prehybridized, and hybridized with riboprobes as described previously (15). Alternatively, the cytoplasmic RNAs were spotted onto Hybond-N membrane in a buffer containing 1.5 M ammonium acetate (using the Schleicher & Schuell Co. slot-blot apparatus and the manufacturer's instructions), and the spotted RNAs were probed as the electroblotted RNAs.

Preparation of cytoplasmic extracts. Cytoplasmic extracts were prepared from uninfected and LAC-infected BHK-21 cells (12 h postinfection [p.i.]) by gentle homogenization of 1 ml of packed cells in 1 ml of buffer containing 10 mM Tris hydrochloride (pH 7.8)–10 mM KCl–1 mM $MgCl_2$ in a Dounce vessel. After disruption the extract was adjusted to 100 mM KCl and centrifuged at 4,000 × *g* to remove the nuclei. The supernatant thus obtained was spun at 15,000 rpm in an SS35 rotor, and the clear cytoplasmic supernatant obtained was frozen in working samples.

Determination of virus released. The amount of virus released from the infected cells was determined by removing 4 ml of infected medium at the various times indicated, preclarifying the medium by centrifuging for 10 min at 10,000 rpm, and then centrifuging at 59,000 rpm in a SW60 rotor for 1 h through a 0.5-ml cushion of 30% glycerol. The pelleted virus samples were then phenol extracted. Half of the viral RNAs thus obtained were analyzed by slot-blot hybridization with a positive-sense riboprobe representing nucleotides 196 through 764 of the LAC S genome and quantitated by densitometry.

RESULTS

LAC infection induces the turnover of intracellular mRNAs. BHK cells were infected with LAC and pulse-labeled with [^{35}S]methionine for 1-h intervals at various times. When total incorporation of methionine into protein was followed, a slight decrease was noted during the first 4 h of the

* Corresponding author.

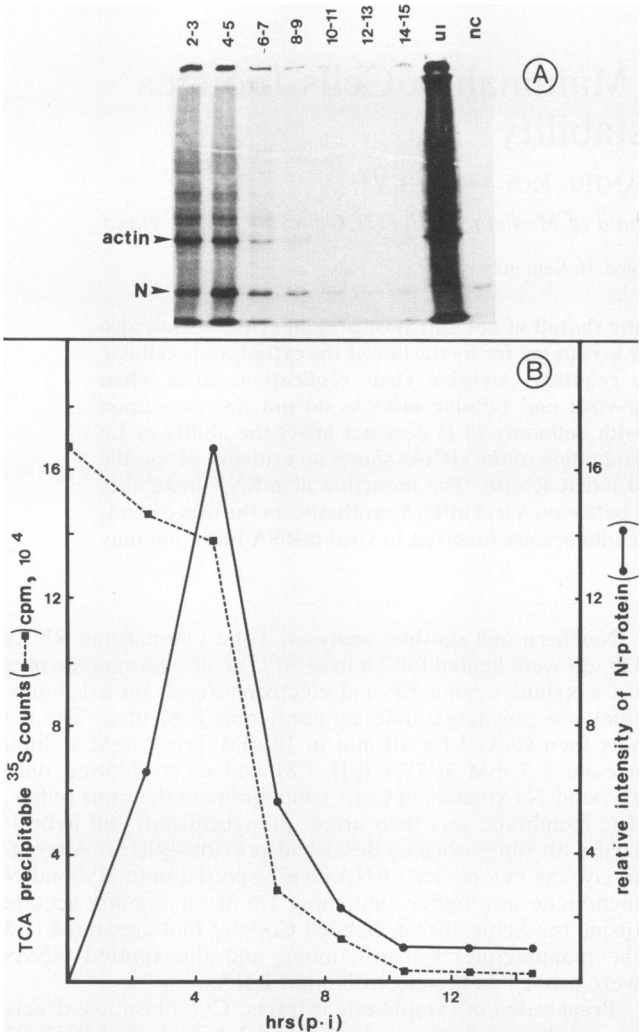

FIG. 1. Inhibition of protein synthesis in LAC-infected cells. (A) Replicate cultures of LAC-infected BHK-21 cells were pulsed with 60 μCi of [^{35}S]methionine for 1 h at the times indicated, and the cultures were harvested at the end of the pulse period. Cytoplasmic extracts were prepared, and 10% of the extracts were separated by sodium dodecyl sulfate–12.5% polyacrylamide gel electrophoresis and fluorographed. The pulse periods are shown (in hours) at the top of each lane. UI, Uninfected culture; nc, N protein isolated from CsCl density gradient-banded nucleocapsids. (B) The extracts were analyzed for their RNase A-resistant, trichloroacetic acid-insoluble counts per minute present in 10% of the extracts. The relative amounts of the viral N protein labeled were determined by densitometric scanning of the lanes shown in A.

infection, but then incorporation was found to decrease precipitously such that it was almost undetectable by 10 h p.i. (Fig. 1B). When the various samples were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, similar results could be seen by examining the host protein background (Fig. 1A), but it is also clear that the viral N protein is made most abundantly at 4 h p.i. and that its synthesis then decreases with kinetics identical to that of the host proteins. When these samples were separated on 17.5% acrylamide gels, the nonstructural protein from the S genome segment could also be detected. Its synthesis paralleled that of the N protein (data not shown); however, the viral glycoproteins and the L protein were too weak to be visible.

Since it is unusual for a major structural component of a virus such as the N protein to be made only relatively early in the infectious cycle, we examined the steady-state concentration of the N-protein mRNA, i.e., the S mRNA, in BHK cells throughout the cycle. Cytoplasmic extracts of the infected cells were first separated by centrifugation through CsCl density gradients into pelleted RNA, which contains the mRNA, and nucleocapsid band material, which contains the encapsidated genomes and antigenomes. The steady-state concentration of the unencapsidated S mRNA was determined; it increased rapidly until 4 h p.i. but then decreased equally rapidly after 6 h p.i. (Fig. 2A). Also shown in Fig. 2 are the extent of CPE in the cultures and the amount of virus liberated into the medium as determined by measuring its S minus-strand genome RNA. The onset of the loss of intracellular S mRNA entirely preceded the appearance of CPE, and virus continued to accumulate in the medium even though mRNA levels and protein synthesis were severely reduced. At 20 h p.i., the vast majority of the cells were still attached to the dish, but they had all rounded up.

The loss of N-protein synthesis after 4 h p.i. can probably be accounted for solely by the loss of S mRNA (Fig. 1 and 2). We therefore examined whether the shutoff of host protein synthesis could be accounted for similarly. CsCl-pelletted RNA from LAC-infected BHK cells harvested at different times was examined for both the viral S mRNA and actin mRNA by using riboprobes. Actin mRNA was unchanged for the first 2 h but then began to decrease in amount, such that at 12 h p.i. it was only 3% of its level at the start of the infection (Fig. 3). The S mRNA showed a pattern of accumulation similar to that in Fig. 2 and did not begin to decrease until 6 h p.i. (Fig. 3A). Both histone mRNA and ribosomal protein L7 mRNA also decreased with the same kinetics as actin mRNA (data not shown). The shutoff of host protein synthesis induced by LAC infection of BHK cells can thus also be accounted for by the loss of the cellular mRNAs.

We also examined whether LAC infection induced the instability of mRNA in other mammalian cell lines. In HeLa, Vero, and HEL cells, S mRNA accumulated to maximal levels by 6 h p.i. and then began to decline in all cases by 12 h p.i. (Fig. 2). LAC infection of Vero, HeLa, and HEL cells was also slower than in BHK cells as judged by both the appearance of CPE and the release of virus into the medium. The induction of mRNA instability is thus not particular to BHK cells but occurs more rapidly and to a greater extent here than in the other cells tested, presumably because LAC replicates more quickly in BHK cells. Further, when LAC infections of BHK cells are found where virus replication is delayed relative to the experiment shown here, then actin mRNA turnover is also delayed similarly (data not shown). It thus appears that LAC S mRNA and host cell mRNA are turned over by a similar if not identical mechanism.

LAC mRNA synthesis continues after induction of mRNA instability. LAC mRNAs initiate on capped primers derived from host cell mRNAs (2, 11), and purified virions contain a methylated cap-dependent endonuclease, presumably for this function (11). The turnover of intracellular mRNA induced by the viral infection could then lead to a suppression of new mRNA synthesis as well. The degraded mRNAs, however, might still serve as substrates for the viral endonuclease, since S mRNA synthesis from purified virions incubated in the presence of a rabbit reticulocyte lysate, which also serves as a source of the primer, is not affected by micrococcal nuclease pretreatment of the lysate (1). To determine whether S mRNA synthesis was also shut off

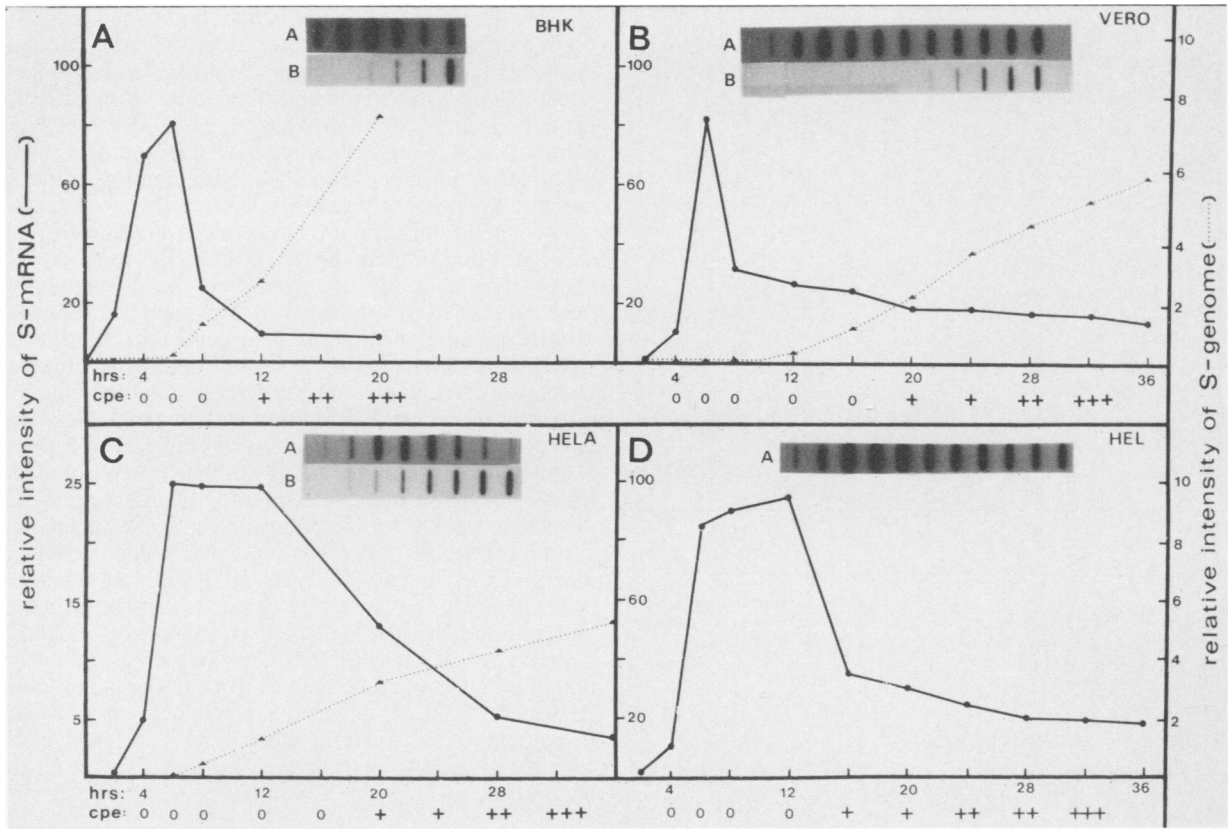

FIG. 2. Steady-state levels of intracellular LAC S mRNA during the infectious cycle. Two petri dishes each of LAC-infected BHK, Vero, HeLa, and HEL cells were harvested at the times indicated, and 20 μg of their CsCl-pelleted RNAs was analyzed by slot-blot hybridization (inset A) by using a negative-sense riboprobe representing nucleotides 196 through 764 of the LAC S genome. The amount of hybridization was quantitated by densitometry and is plotted on an arbitrary scale. The scale for HeLa cells has been amplified fourfold relative to the others. The amount of virus released from the infected cells was determined by measuring its S genome, also by hybridization (inset B; see Methods and Materials). The extent of CPE induced by the infection is represented by + signs, + + + + being maximal. The slot blots in insets A and B are ordered with the first time point on the left and the last on the right.

during infection, we pulse-labeled infected BHK cultures with [^{3}H]uridine for 15 and 45 min at different times p.i. and determined the amount of ^{3}H-RNA in the CsCl pellet fraction that was protected from RNase digestion after annealing with an excess of minus-strand RNA. The amount of S mRNA labeled in the relatively long pulse time of 45 min (Fig. 4) mirrored that of mRNA accumulation (Fig. 2 and 3), whereas that labeled in the 15-min pulse did not decrease as sharply after 6 h. The ratio of the S mRNA labeled in 45- to 15-min pulses was a factor of more than 4 at 3 h p.i., when little or no mRNA instability would be expected, but declined to almost 1 by 11 h p.i. (Fig. 4). At 11 h p.i. S mRNA was still being labeled in a 15-min pulse at 55% of the amount as at 6 h, when mRNA accumulation is maximal, but its true rate of synthesis is almost certain to be higher because of mRNA turnover even within 15 min.

LAC S-mRNA synthesis thus continued in infected cells even when both cellular and viral mRNAs were being rapidly turned over and protein synthesis had been mostly turned off. However, a low level of N protein synthesis was still visible even at 12 to 20 h p.i., when it was the only band visible by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 1A). This low level presumably reflects, at least in part, translation of the S mRNA as it is being made and before it is released from its template.

Induction of mRNA instability requires more than 2 h of infection. LAC S mRNA synthesis is known to be unusually sensitive to drugs that disrupt protein synthesis (13). In the absence of protein synthesis, S mRNA synthesis initiates normally but terminates prematurely (1, 14). Complete mRNA synthesis can therefore be interrupted at any time p.i. by drug treatment, and the fate of the preexisting mRNA can be followed more clearly. We treated infected cultures with cycloheximide relatively early in infection (2 h p.i.), when S mRNA accumulation was just beginning, or at 6 h p.i., when accumulation had peaked, and then determined the steady-state concentration of S mRNA by Northern (RNA blot) analysis and of actin mRNA by slot-blot analysis (Fig. 3). When cycloheximide was added at 2 h p.i., the S mRNA that had been made before drug addition and the actin mRNAs did not decrease in amount but remained stable until 20 h. However, when the drug was added at 6 h p.i., both S mRNA and actin mRNA levels decreased similarly to the untreated control cultures. Since ongoing protein synthesis was not required for mRNA turnover when cycloheximide was added at 6 h p.i., the lack of mRNA turnover when the drug was added at 2 h p.i. can only mean that mRNA instability had not been induced by this time. At 2 h p.i., genome amplification had clearly begun, but genomes and antigenomes had accumulated to only 10 to 15% of the maximal level. At 6 h p.i., genomes and antigenomes had accumulated to 95% of their maximal levels (14). The induction of mRNA instability thus requires viral replication to near-maximal levels.

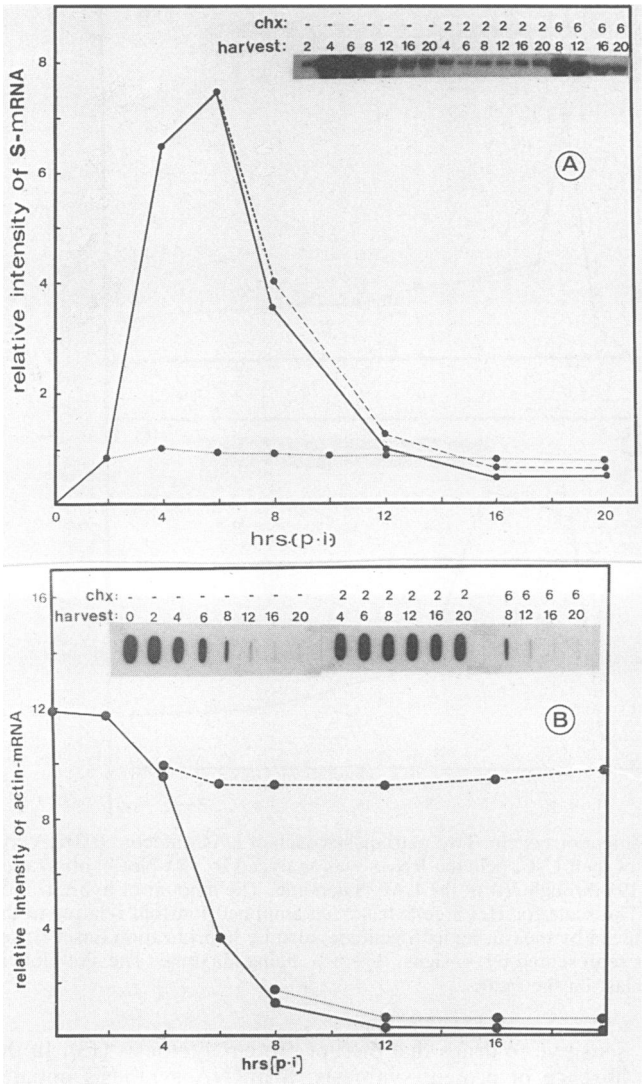

FIG. 3. Effect of cycloheximide (chx) on mRNA instability in LAC-infected cells. (A) Three series of BHK-21 cultures were infected with LAC virus. To the first series no drug was added (——). The second series of cultures were treated with 50 μg of cycloheximide per ml at 2 h p.i. (. . . .), and the third series of cultures were similarly treated at 6 h p.i. (– – –). Groups of four petri dishes were harvested at the times indicated, and 5% of their CsCl pelleted RNAs were analyzed on Northern blots (inset) of a 4% polyacrylamide gel and analyzed as in Fig. 1. (B) The same RNA samples were analyzed by slot-blot hybridization with a 320-nucleotide-long negative-sense riboprobe of a rat actin cDNA clone. Densitometric scanning of the autoradiograms was used to quantify the results. The numbers above the autoradiogram inset refer to the time p.i. in hours. Symbols: cycloheximide treatment at 2 h p.i. (– – –) and at 6 h p.i. (. . . . .).

LAC induction of mRNA instability is independent of the 2-5 A-activated pathway. LAC infection of BHK cells may induce the interferon-mediated antiviral state. One enzyme induced by interferon which could well be responsible for mRNA instability is 2-5 oligoriboadenylate (2-5 A) synthetase, whose product serves as a cofactor for a latent RNase that is known to degrade mRNAs (6). If the induction of mRNA instability by LAC infection were the result of the infection itself inducing the antiviral state, then these effects of IFN should be abolished if infection took place in cells pretreated with actinomycin D, so that no new host mRNA synthesis could take place (6). We therefore examined the effect of pretreating cells before infection with actinomycin D on the stability of S mRNA after LAC infection. Pretreatment of cells with actinomycin D did not prevent the S mRNA from turning over with kinetics identical to that in the untreated control cultures (Fig. 5A).

The inability of actinomycin D to prevent mRNA instability argues that no new mRNA synthesis is required for this effect. It is possible, however, that the 2-5 A synthetase mRNA is constitutively expressed in our BHK cells, but the enzyme remains inactive until the viral infection generates sufficient double-stranded RNA, an obligatory cofactor. In this case, LAC induction of mRNA instability could still be the result of the 2-5 A activation of the latent RNase even in the absence of new host mRNA synthesis. We therefore examined whether there was any other evidence of the latent RNase having been activated upon LAC infection. The most diagnostic marker for the latent RNase is the specific degradation of rRNA (18). However, when the rRNAs from mock-infected and 10-h infected BHK cells were compared, no evidence of any specific degradation of the rRNA could be found in cells in which both viral and host mRNAs had largely turned over (Fig. 5B). This lack of specific degradation of rRNA is not due to an absence of the latent RNase in BHK cells, since the addition of nanomolar amounts of 2-5 A to cytoplasmic extracts of mock- and LAC-infected BHK cells led to specific degradation of the rRNA, but equally in both extracts (Fig. 5B). Taken together, these experiments

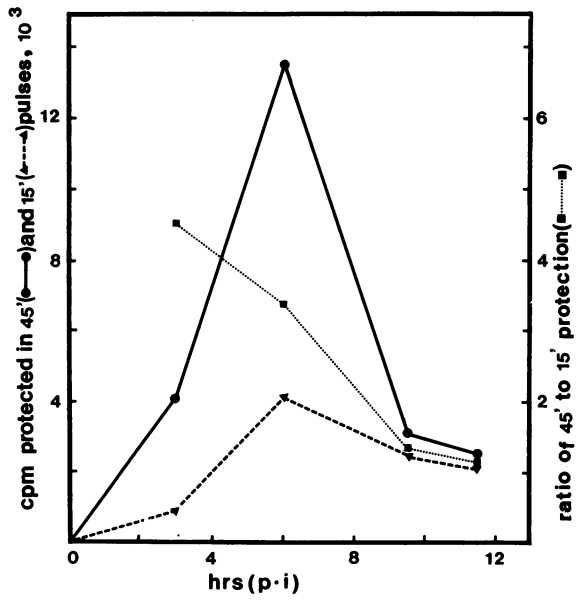

FIG. 4. Rate of synthesis of LAC S-mRNA during the replication cycle. BHK-21 cells were infected with LAC, and replicate cultures were pulsed with 300 μCi of tritiated uridine per plate for 45 (——) or 15 (– – –) min at the times indicated. At the end of the pulse period the cultures were harvested, and equal amounts of the CsCl-pelleted RNAs were annealed to an estimated threefold and sixfold excess of nonradioactive negative-sense riboprobes representing nucleotides 1 through 764 and then digested with pancreatic RNase as previously described (14). A background of 186 cpm from control samples annealed with tRNA has been subtracted. The ratio of the protected counts per minute in the 45- to 15-min pulses is also shown (. . . . .).

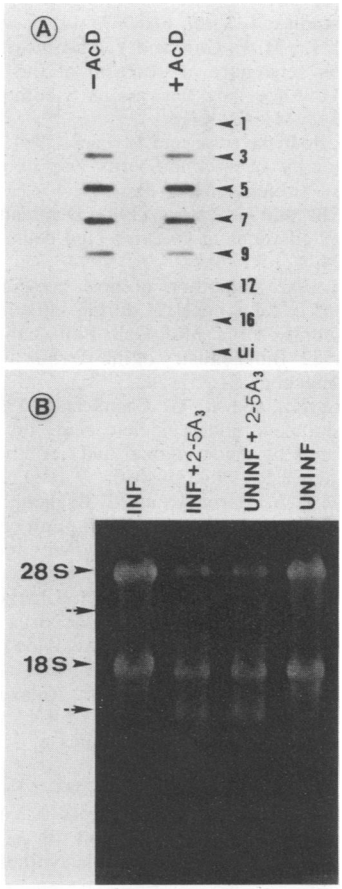

FIG. 5. The 2-5 A pathway is not involved in LAC-induced mRNA instability. (A) mRNA instability does not require host transcription. Two series of BHK-21 cultures were infected with LAC either in the absence (− AcD) or in the presence (+ AcD) of 5 μg of actinomycin D per ml. Two petri dishes each were harvested at the times indicated, and 5% of their CsCl pelleted RNAs were analyzed by slot-blot hybridization as in Fig. 1. The numbers refer to hours p.i. ui, uninfected control RNA. (B) Effect of 2-5 ribotriadenylate on the rRNA integrity of uninfected and LAC-infected BHK-21 cellular extracts. Samples (50 μl) of the extracts were incubated either in the absence or in the presence of 150 nM 2-5 ribotriadenylate at 30°C for 30 min. The extracts were phenol extracted; the RNAs were ethanol precipitated, denatured with glyoxal, separated on a 1.2% agarose gel in 10 mM sodium phosphate buffer (pH 7) (7), and stained with ethidium bromide. The oligo(A)-induced cleavage products are marked by arrows. Unincubated extracts showed identical results to those incubated in the absence of 2-5 ribotriadenylate.

indicate that LAC induction of mRNA instability is not the result of 2-5 A activation of the latent RNase.

DISCUSSION

Both LAC N protein and host protein synthesis decrease rapidly 4 to 6 h p.i. in BHK cells, and virtually identical results have previously been reported for bunyamwera virus (another member of the *Bunyavirus* genus) in BSC-1 cells. This shutoff of both viral and host protein synthesis can be accounted for largely, if not entirely, by the loss of the intracellular mRNAs. When actin mRNA levels in BHK cells were followed as an example of a host mRNA, they decreased to 10% of their amount at the start of the infection by 8 h and to 3% by 12 h p.i. Viral S mRNA accumulation increased until 4 to 6 h p.i. (this was reflected by the rate of N-protein synthesis) but then decreased with kinetics similar to that of host mRNA. Using cycloheximide treatment, which inhibits LAC mRNA synthesis as well as protein synthesis, we could then follow the fate of preexisting mRNA. We found that when the drug was added at 2 h p.i. the activity responsible for S mRNA or actin mRNA instability had not as yet been induced, but by 6 h p.i. this activity appeared to have accumulated to maximum levels. The induction of mRNA instability therefore requires more than just primary transcription and translation and the onset of genome replication. Since CPE was not visible until 12 h p.i. and virus continued to be released into the medium at least until 20 h p.i., this loss of intracellular mRNA does not appear to be due to the CPE of the infection, but rather the CPE is at least in part a consequence of the loss of mRNA.

When the induction of mRNA instability was first noted, it seemed that the most logical explanation would be the activation of the interferon-associated latent RNase by the viral infection. Most, if not all, animal cells express this RNase constitutively, but its activity depends on the presence of 2-5 A. The enzyme responsible for 2-5 A synthesis is not present under normal conditions but is one of the enzymes induced by interferon. This induction by interferon, however, normally requires transcription of the 2-5 A synthetase mRNA. Our finding that the induction of mRNA instability by LAC infection is unaltered by actinomycin D pretreatment of the cultures suggests that this effect is unlikely to be mediated through an interferon pathway. Further, examination of the rRNAs from uninfected and LAC-infected cells showed no evidence that the latent RNase had been activated during infection, even though the enzyme could be demonstrated in extracts of uninfected and infected cultures. Curiously, we have not been able to demonstrate the LAC-induced nuclease in these same extracts by using either capped or uncapped SP6 derived transcripts (data not shown).

The nuclease induced by LAC infection degrades mRNAs, either viral or host, but does not degrade rRNA, unlike the interferon-associated latent RNase in cells infected with reovirus (8), encephalomyocarditis virus (17), or vaccinia virus (3, 4). One possibility is therefore that the nuclease recognizes a structure common to mRNAs, e.g., the cap group. We have previously shown that purified LAC virions contain a methylated cap-dependent endonuclease, which cleaves the cap and the following 10 to 15 nucleotides from mRNAs for their subsequent use as primers for mRNA synthesis (10). In vitro, this endonuclease can cleave exogenous mRNA in the absence of mRNA synthesis, and so cleavage is not necessarily coupled to viral mRNA synthesis. This endonuclease, presumably a function of the viral polymerase (the L protein), is therefore a possible candidate for this mRNA-specific turnover activity, and the kinetics of induction of mRNA instability fits nicely with the accumulation of viral proteins. The loss of its capped 5′ end would then cause the mRNAs to become labile to exonucleases, leading to their disappearance as judged by slot-blot and Northern analyses. Our inability to find even trace amounts of the intermediates in mRNA breakdown by Northern analysis (data not shown) is consistent with this hypothesis. However, the viral endonuclease would have to cleave its own mRNA at a rate comparable to that of cellular mRNA. Although there is no a priori reason why this cannot be true in animal cells, we have little or no evidence that any of the S-genome transcripts made in vitro have lost their host mRNA-derived primer.

Other animal viruses such as vaccina (9), herpes simplex (16), and influenza (5) viruses shut off host protein synthesis in some cells by degrading host mRNAs in the cytoplasm, but in these infections late viral protein synthesis is not inhibited. In the case of vaccinia virus the turnover of host mRNAs appears to be due to the 2-5 A-activated latent RNase, whereas in herpes simplex virus infections a virion-associated factor has been implicated, since turnover takes place even when protein and RNA syntheses are inhibited from the onset of infection. The mechanism of mRNA turnover in influenza virus infection of chicken embryo fibroblasts is unknown; with this possible exception, the various virus-induced turnovers of host mRNAs appear to take place by different mechanisms. What is peculiar to LAC infections of mammalian cells is that viral and host mRNAs are equally susceptible to turnover. However, this need not represent a fundamental mechanistic difference from the other viruses. Since pulse-labeling of infected cells with [^{3}H]uridine indicates that viral mRNA synthesis has not been shut off, the bell-shaped curve of S mRNA accumulation would appear to be the result of an increasing rate of its turnover relative to its synthesis. The reason that host and not viral protein synthesis is inhibited in the vaccinia and influenza virus infections may then simply by that the rate of synthesis of these viral mRNAS always exceeds that of the turnover activity, rather than that the turnover activity can distinguish between viral and host mRNAs. In this case, the turnover of viral and host mRNAs by the LAC infection is presumably due to the induction of a nuclease (either viral or host) which is so powerful that it overcomes even the accumulation of the viral mRNAs.

LAC infection of mosquito cells, its natural host, on the other hand is completely asymptomatic; the infected cells continue to divide normally as a persistent infection. In mosquito cells, the nuclease either is not induced if it is of cellular origin or somehow is kept under control by a mechanism that does not operate in mammalian cells if it is of viral origin. It is worth noting in this context that mammals play no role that is essential to the survival of this virus in the natural history of LAC infections, including that of overwintering (19, 20). The cytopathic effect of mRNA turnover seen in animal cells might therefore not have been important in the evolution of this virus and its adaptation to its natural host.

ACKNOWLEDGMENTS

The expert technical assistance of Rosette Bandelier and Catherine Stouder is gratefully acknowledged. We thank Daniel Schumperli, Olivier Kocher, and Ueli Schibbler for providing us with DNA clones for mouse histone, rat actin, and mouse ribosomal protein L7 mRNAs, respectively.

This work was supported by a grant from the Swiss National Science Foundation.

LITERATURE CITED

1. **Bellocq, C., R. Raju, J. Patterson, and D. Kolakofsky.** 1987. Translational requirement of La Crosse virus S-mRNA synthesis: in vitro studies. J. Virol. **61**:87–95.
2. **Bishop, D. H. L., M. E. Gay, and Y. Matsuoko.** 1983. Nonviral heterogeneous sequences are present at the 5′ ends of one species of showshoe hare bunyavirus S complementary. Nucleic Acids Res. **11**:6409–6418.
3. **Esteban, M., J. Benaventa, and E. Paez.** 1984. Effect of interferon on integrity of vaccinia virus and ribosomal RNA in infected cells. Virology **134**:40–51.
4. **Goswami, B. B., and K. Sharma.** 1984. Degradation of rRNA in interferon-treated vaccinia virus-infected cells. J. Biol. Chem. **259**:1371–1374.
5. **Inglis, S. C.** 1982. Inhibition of host protein synthesis and degradation of cellular mRNAs during infection by influenza and herpes simplex virus. Mol. Cell. Biol. **2**:1644–1648.
6. **Lengyel, P.** 1982. Biochemistry of interferons and their actions. Annu. Rev. Biochem. **51**:251–282.
7. **McMaster, G. K., and G. G. Carmichael.** 1977. Analysis of single- and double-stranded nucleic acids on polyacrylamide and agarose gels by using glyoxal and acridine orange. Proc. Natl. Acad. Sci. USA **74**:4835–4840.
8. **Nilsen, T. W., P. A. Maroney, and C. Baglioni.** 1982. Synthesis of (2′-5′) oligoadenylate and activation of an endoribonuclease in interferon-treated HeLa cells infected with reovirus. J. Virol. **42**:1039–1045.
9. **Pacha, R. F., and R. C. Condit.** 1985. Characterization of a temperature-sensitive mutant of vaccinia virus reveals a novel function that prevents virus-induced breakdown of RNA. J. Vi rol. **56**:395–403.
10. **Patterson, J. L., B. Holloway, and D. Kolakofsky.** 1984. La Crosse virus contain a primer-stimulated RNA polymerase and a methylated cap-dependent endonuclease. J. Virol. **52**:215–222.
11. **Patterson, J. L., and D. Kolakofsky.** 1984. Characterization of La Crosse virus small-genome transcripts. J. Virol. **49**:680–685.
12. **Pennington, T. H., C. R. Pringle, and M. A. McCrae.** 1977. Bunyamwera virus-induced polypeptide synthesis. J. Virol. **24**: 397–400.
13. **Raju, R., and D. Kolakofsky.** 1986. Inhibitors of protein synthesis inhibit both La Crosse virus S-mRNA and S genome synthesis in vivo. Virus Res. **5**:1–9.
14. **Raju, R., and D. Kolakofsky.** 1987. Translational requirement of La Crosse virus S-mRNA synthesis: in vivo studies. J. Virol. **61**:96–103.
15. **Raju, R., and D. Kolakofsky.** 1987. Unusual transcripts in La Crosse virus-infected cells and the site for nucleocapsid assembly. J. Virol. **61**:667–672.
16. **Schek, N., and S. L. Bachenheimer.** 1985. Degradation of cellular mRNAs induced by a virion-associated factor during herpes simplex virus infection of vero cells. J. Virol. **55**:601–610.
17. **Silverman, R. H., J. Cayley, M. Knight, C. S. Gilbert, and I. M. Kerr.** 1982. Control of the ppp(A2′p)$_n$A system in HeLa cells. Effects of interferon and virus infection. Eur. J. Biochem. **124**:131–138.
18. **Silverman, R. H., J. J. Skehel, T. C. James, D. H. Wreschener, and I. M. Kerr.** 1983. rRNA cleavage as an index of ppp(A2′p)$_n$A activity in interferon-treated encephalomyocarditis virus-infected cells. J. Virol. **46**:1051–1055.
19. **Thompson, W. H.** 1983. Vector-virus relationships, p. 57–66. *In* California serogroup viruses. Alan R. Liss, Inc., New York.
20. **Turell, M. J., and J. W. LeDuc.** 1983. The role of mosquitoes in the natural history of California serogroup viruses, p. 43–55. *In* California serogroup viruses. Alan R. Liss, Inc., New York.