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PROTEINS ASSOCIATED WITH mRNA IN CELLS INFECTED WITH HERPES SIMPLEX VIRUS

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The structure of messenger ribonucleoprotein (mRNP) complexes in herpes simplex virus type 1 (HSV-1) infected cells was analyzed by examining the proteins that could be crosslinked to polyadenylated mRNAs by irradiation of intact cells with ultraviolet light. The profiles of crosslinked proteins were qualitatively similar for mRNPs from mock infected and infected cells. However, infection with wild type HSV-1 caused a decrease in the abundance of a major 52 kda protein and an increase in a 49 kda protein. These changes were observed at early times after infection. They occurred following infection with wild type HSV-1 under conditions that blocked viral gene expression, but not following infection with the virion host shutoff mutant vhs 1.

In the cytoplasm of mammalian cells, mRNA molecules are found associated with specific proteins in structures known as messenger ribonucleoprotein (mRNP) complexes (1-4). The functions of these proteins remain largely unknown, although the locations within the complex of several have been identified. A 72 kilodalton (kda) protein is known to associate with the poly(A) tail (5,6), while a number of others bind to the 5' cap structure (7). A 50 kda major mRNP protein has been reported to associate with both the 5' cap structure and the body of the mRNA, but not with the poly(A) tail (7). Possible functions for these proteins include transport of the mRNA, control of mRNA stability, and regulation of translation.

One of the earliest events during lytic infections with herpes simplex virus, type 1 (HSV-1) is the shutoff of most cellular protein synthesis (8-14). This is caused by a structural component of the infecting virion, the virion host shutoff (vhs) protein, which induces degradation of host mRNAs (11,13,15,16). The vhs protein is not specific for cellular mRNAs, since, following the onset of viral gene expression, it also causes rapid turnover of viral mRNAs (10,17,18). Thus, the mutant vhs 1, which encodes a defective vhs protein, is defective in virion host shutoff (13) and produces viral mRNAs with significantly longer half lives (17,18).

Since factors that affect mRNP structure or composition could affect the stabilities of mRNAs, we examined the proteins associated with mRNA in HSV-1

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infected cells using an approach that involves ultraviolet light-induced crosslinking of proteins to mRNA in intact cells. We report that although the protein components of mRNPs were similar in infected and mock infected cells, wild type HSV-1 infection led to several quantitative changes in mRNP composition at early times after infection. These changes were induced by a virion component, and correlated with a wild type vhs activity.

MATERIALS AND METHODS

Cells and virus. HeLa S3 cells were grown and infected with wild type HSV-1, strain KOS, or the mutant vhs 1 as described previously (18). Mock infected cells were treated in the same way as infected cells, except that they were exposed to lysates of uninfected Vero cells prepared in the same way as the virus stocks were prepared from infected cells.

UV irradiation and mRNP preparation. HeLa cells were grown to subconfluent densities and labeled for the intervals described in the text by exposure to Eagle's Minimum Essential Medium containing one-tenth the normal amount of methionine, 20 uCi/ml [^{35}S]methionine, and 5% calf serum.

Ultraviolet light irradiation and mRNP isolation were performed essentially as described by Adam and Dreyfuss (6). Cell monolayers were washed with ice-cold phosphate buffered saline (PBS), and then exposed for three minutes to a 30 watt germicidal lamp (Sylvania G30T8) placed 4.5 cm from the monolayer surface. After irradiation, the cells were allowed to swell in ice-cold RSB (10 mM Tris HCl, pH 7.4; 10 mM NaCl; 1.5 mM $MgCl_2$) containing 0.5% aprotinin (Sigma), 1 ug/ml leupeptin (Sigma), 1 ug/ml pepstatin (Sigma), and 10 mM vanadyl ribonucleoside complex (VRC; Bethesda Research Laboratories). They were then lysed by the addition of Triton X-100 to 0.5%, Tween 40 to 1%, and sodium deoxycholate to 0.5% followed by homogenization by four passes through a 25-gauge needle. The nuclei were pelleted by low speed centrifugation, and the supernatant was considered the cytoplasmic fraction.

This fraction was adjusted to 0.5% SDS, 1% 2-mercaptoethanol, and 10 mM EDTA, heated to 65 ^{o}C for five minutes, and then chilled on ice. LiCl was added to 0.5 M, and polyadenylated mRNPs were selected by chromatography of the cytoplasmic mixture over columns of oligo(dT)-cellulose (type 3; Collaborative Research). The eluted material was concentrated by ethanol precipitation, and the RNA moiety was then degraded by digestion with RNase A and micrococcal nuclease (6). The protein components of the mRNPs were then analyzed as described previously (17) by electrophoresis through gels of 12% acrylamide, 0.3% DATD and 0.1% SDS and autoradiography.

RESULTS

To examine the proteins associated with mRNA in HSV-1 infected cells we used the uv crosslinking approach developed by Dreyfuss (1) and others (2-4). HeLa cells were prelabeled with [^{35}S]methionine for 4 h. immediately prior to mock infection or infection with 20 pfu/cell of wild type HSV-1. The cells were then incubated in the absence of label from 0 to 3 h. post infection, at which time mRNPs were prepared. At least seven protein bands could be resolved in mRNPs from mock infected cells (Figure 1A, lane 1). Prominent bands were seen at 125, 73, and 52 kda, with minor bands at 49, 39, 34, and 32 kda. The 73 kda protein has been shown to be the major cytoplasmic poly(A) binding protein (5,6). Isolation of these proteins as part of mRNPs was depe-

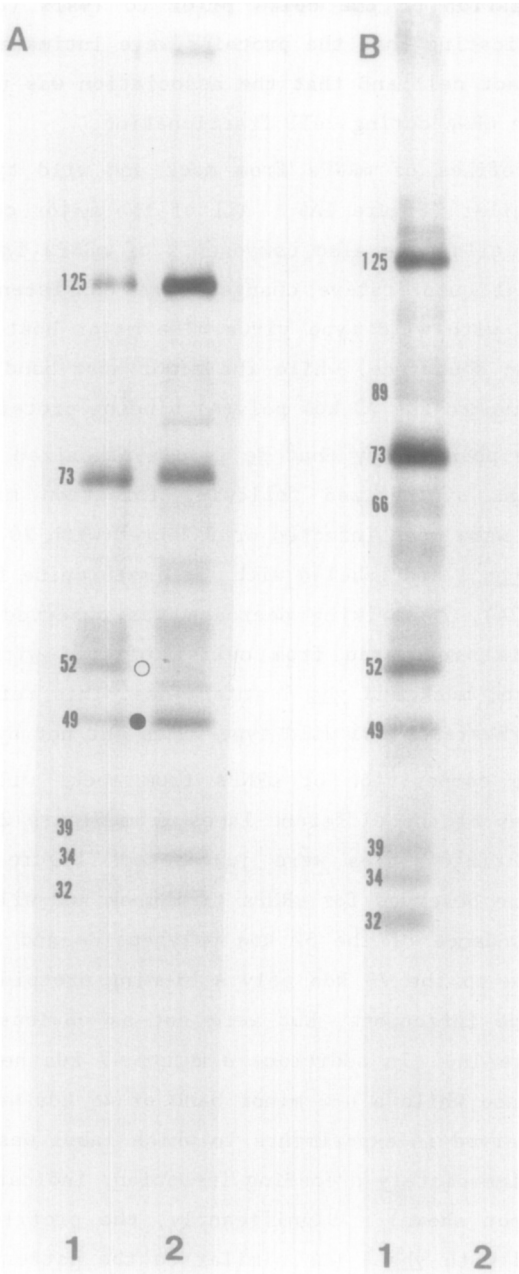


Figure 1. mRNPs from infected and mock infected cells. A. HeLa cells were prelabeled for 4 h. with [^{35}S]methionine and then mock infected (lane 1) or infected with 20 pfu/cell of wild type HSV-1 (lane 2). The cells were incubated in the absence of label from 0 to 3 h. post infection, at which time mRNPs were prepared and analyzed. The molecular weights (in kilodaltons) of prominent proteins are shown to the left of lane 1. The 52 kda polypeptide whose abundance is decreased by wild type virus infection is marked by an open circle to the right of lane 1, while the 49 kda protein that is increased in abundance is labeled by a closed circle. B. Parallel cultures were labeled as in part A and infected with 20pfu/cell of wild type HSV-1. At 3 h. post infection the cells were either irradiated with u.v. light (lane 1) or not irradiated (lane 2). The cells were then lysed and mRNP samples prepared according to the remainder of the normal protocol described in the text.

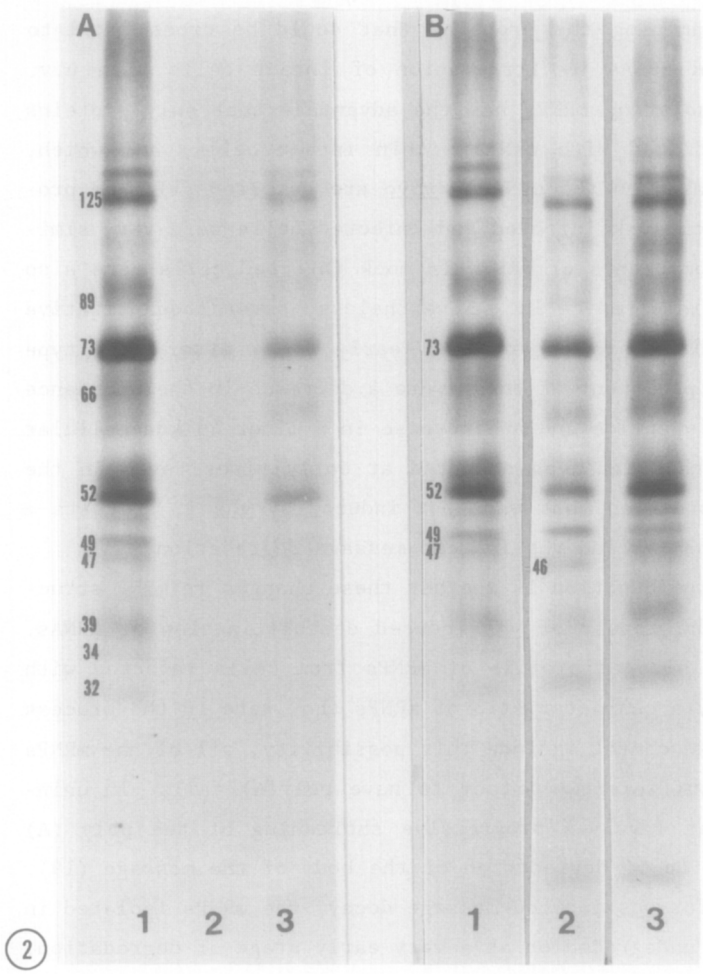
pendent upon irradiation of the cells prior to lysis (compare Figure 1B, lanes 1 and 2), indicating that the proteins were intimately associated with mRNA within the intact cell and that the association was not due to artifactual sticking to the mRNA during cell fractionation.

The protein profiles of mRNPs from mock and wild type virus infected cells were very similar (Figure 1A). All of the major components of mRNPs from mock infected cells were also components of mRNPs from infected cells. Nevertheless, several quantitative changes were consistently seen in mRNPs from cells infected with wild type virus. The major host polypeptide of 52 kda was decreased in abundance, while the minor host band of 49 kda was increased, both relative to the 73 kda poly(A) binding protein.

To examine the possibility that de novo synthesized viral polypeptides or cellular proteins synthesized following infection might be mRNP components, HeLa cells were mock infected or infected with 20 pfu/cell of either wild type HSV-1 or vhs 1 and labeled with [^{35}S]methionine from 0 to 3 h. post infection (Figure 2A). A striking decrease was observed in the amount of labeled mRNP material recovered from cells infected with wild type virus compared to that from mock and vhs 1 infections. This reflected the shutoff of host protein synthesis by the wild type virus but not by vhs 1.

To compare the composition of mRNPs from mock, wild type and vhs 1 infections, exposures of the different lanes from Figure 2A that resulted in approximately equal intensities were juxtaposed (Figure 2B). Once again, similar profiles were observed for mRNPs from mock and wild type infections. The decrease in abundance of the 52 kda polypeptide and increase in the 49 kda protein relative to the 73 kda poly A binding protein were observed for mRNPs from wild type infections, but were not as obvious in these gel exposures as in Figure 1A. In addition, a minor 47 kda host polypeptide was decreased in abundance while a new minor band of 46 kda was observed. The 46 kda protein was observed in experiments in which label was present only during the four hours immediately preceding infection, indicating that it was of host origin (data not shown). Significantly, the profile of mRNP proteins from cells infected with vhs 1 was similar to the pattern from mock infections, and did not show the changes characteristic of wild type virus infections. Furthermore, no de novo synthesized viral proteins were detected in mRNPs from either wild type or vhs 1 infections.

To test the apparent correlation between changes in mRNP composition and vhs activity, we determined whether the changes induced by wild type virus still occurred following infections under conditions that prevented viral gene expression. Cells were prelabeled with [^{35}S]methionine for 4 h. and then mock infected or infected with 50 pfu/cell of either wild type virus or vhs 1 in the presence of 5 ug/ml of actinomycin D. Wild type virus induced



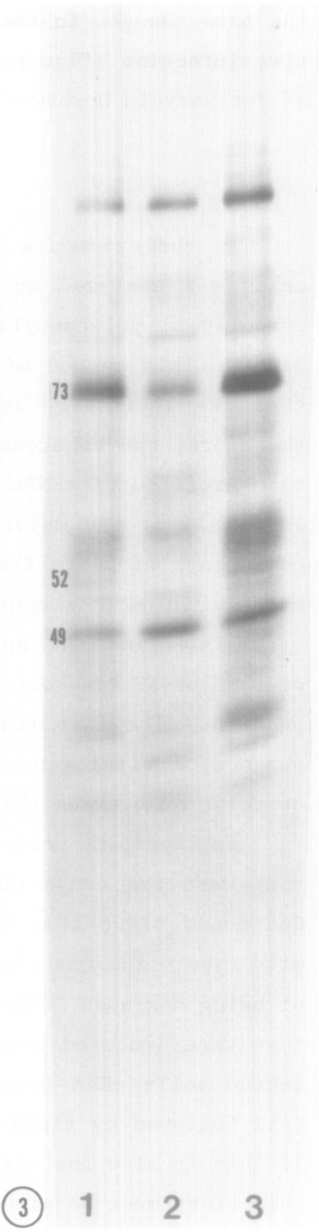


Figure 2. mRNPs proteins synthesized following infection or mock infection. A. HeLa cells were mock infected (lane 1) or infected with 20 pfu/cell of wild type virus (lane 2) or vhs 1 (lane 3). The cells were labeled with [^{35}S]methionine from 0 to 3 h. post infection, and mRNPs were prepared and analyzed at 3 h. Material from approximately 3 x 10^7 cells was loaded onto each lane of the gel. B. Multiple exposures were obtained for the gel shown in part A, and exposures resulting in similar intensities for the mock (lane 1), wild type (lane 2), and vhs 1 (lane 3) infections were juxtaposed. The molecular weights (in kilodaltons) of prominent proteins are shown to the left of lane 1 in both A and B.

Figure 3. Changes in mRNP structure occur in wild type infections in the absence of viral gene expression. HeLa cells were prelabeled with [^{35}S]-methionine for 4 h. and then mock infected (lane 1) or infected with 50 pfu/-cell of wild type virus (lane 2) or vhs 1 (lane 3), all in the presence of 5 ug/ml of actinomycin D. mRNPs were prepared at 3 h. post infection or mock infection. The molecular weights (in kilodaltons) of prominent proteins are shown to the left of lane 1.

the same changes in the presence of actinomycin D (Figure 3) as in a productive infection (Figures 1 and 2). Cells infected with vhs 1 in the presence of actinomycin D showed the same pattern as mock infected cells (Figure 3).

DISCUSSION

In these studies the composition of mRNP complexes in HSV-1 infected cells was analyzed by examining the proteins that could be crosslinked to cytoplasmic polyadenylated mRNAs by irradiation of intact cells with u.v. light. This method of isolating mRNPs has the advantage that only proteins that are intimately associated with mRNAs within intact cells, and which, therefore, can be crosslinked to the mRNA in vivo are detected (1). The protein profiles of mRNPs from mock infected and infected cells were very similar. All of the major components of mRNPs in mock infected cells were also components of mRNPs from infected cells. Nevertheless, several quantitative changes in mRNP composition were observed at early times after wild type virus infection. The most prominent of these were a decrease in the abundance of a major 52 kda host polypeptide and an increase in a minor 49 kda cellular protein. The fact that these changes occurred at early times, even in the absence of viral gene expression, but were not induced by vhs 1, suggests a correlation between the changes and vhs induced message degradation.

An important unanswered question is whether these changes in mRNP structure were the cause or the result of vhs induced destabilization of mRNAs. One could argue that the altered profile of mRNPs from cells infected with wild type virus was simply a characteristic of mRNPs that were in the process of being degraded. While we cannot exclude this possibility, all of the mRNPs that were isolated were sufficiently intact to have poly(A) tails. In uninfected cells mRNA turnover involves progressive shortening of the poly (A) tail followed by rapid 3′ to 5′ degradation of the body of the message (19). If this is also the case for vhs induced message decay, the mRNPs isolated in this study must be either undegraded or at a very early stage of degradation.

In an earlier report Bartoski suggested that productive infection of HEp-2 cells with HSV-1, strain F, caused a dramatic decrease in the amount of the 73 kda poly(A) binding protein within polysomal mRNPs (20). We did not see such a decrease. In his study Bartoski prepared polyribosomes from infected cells, dissociated them with EDTA, and then selected polyadenylated mRNPs by oligo(dT) chromatography. No u.v. crosslinking was utilized in any step of the procedure. The reasons for the discrepancies between the two studies are unclear, but probably reflect differences in the preparation of mRNPs. One possibility is that Bartoski's mRNPs, with diminished amounts of the poly(A) binding protein, may have represented mRNPs at a later stage in the degradation process than did the mRNPs described in this paper.

The mechanism of vhs action is unknown. The vhs protein may itself be a viral ribonuclease, or it may activate cellular enzymes. Interestingly, the 52 kda polypeptide that is decreased in abundance in wild type infections is probably the protein suggested by Greenberg to be a relatively non-specific RNA binding protein that associates with all portions of the message except the poly(A) tail (7). The decrease in this major protein, therefore, may reflect significant changes in mRNP structure that render the mRNAs more susceptible to cellular ribonucleases. Elucidation of the exact mechanism of vhs action will await experiments using an in vitro mRNA degradation system.

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