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The Influenza Virus NS1 Protein Is a Poly(A)-Binding Protein That Inhibits Nuclear Export of mRNAs Containing Poly(A)

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The influenza virus NS1 protein inhibits the nuclear export of a spliced viral mRNA, NS2 mRNA (F. V. Alonso-Caplen, M. E. Nemeroff, Y. Qiu, and R. M. Krug, Genes Dev. 6:255–267, 1992). To identify the sequence in NS2 mRNA that is recognized by the NS1 protein, we developed a gel shift assay for the formation of specific RNA-protein complexes. With this assay, it was established that the NS1 protein binds to the poly(A) sequence at the 3′ end of NS2 mRNA and of other mRNAs. In addition, the NS1 protein was shown to bind to poly(A) itself. This specificity was also observed in vivo. The NS1 protein inhibited the nuclear export of every poly(A)-containing mRNA that was tested. In contrast, the NS1 protein failed to inhibit the nuclear export of an mRNA whose 3′ end was generated by cleavage without subsequent addition of poly(A). Addition of poly(A) to this mRNA enabled the NS1 protein to inhibit mRNA export. The implications of these results for the role of the NS1 protein during virus infection are discussed.

Posttranscriptional control of eukaryotic gene expression requires the interaction of regulatory proteins with specific RNA sequences. These regulatory proteins include some of the heterogeneous nuclear RNA-binding proteins (7), proteins that regulate splice site selection like the sex lethal protein of *Drosophila melanogaster* and the ASF/SF2 protein (10, 12, 17), and the Rev protein of human immunodeficiency virus, which regulates the transport of unspliced human immunodeficiency virus pre-mRNA (15, 16). Our recent results indicate that the influenza virus NS1 protein is also a posttranscriptional regulator of gene expression (2). We showed that this protein regulates the nuclear export of a viral mRNA in that it inhibits the nuclear export of viral spliced NS2 mRNA.

The present study was undertaken to identify the sequence in the viral NS2 mRNA that is recognized by the NS1 protein. For this purpose we developed a specific in vitro RNA binding assay for the NS1 protein. Surprisingly, by using this assay we found that the NS1 protein specifically binds to the poly(A) sequence at the 3′ end of NS2 mRNA. In fact, the NS1 protein binds to the poly(A) sequence at the 3′ ends of other mRNAs and to poly(A) itself. This in vitro specificity was also shown to be exhibited in vivo. We demonstrate that the NS1 protein inhibits the nuclear export of mRNAs only when they contain poly(A). These results indicate that the NS1 protein would be expected to inhibit the nuclear export of all poly(A)-containing viral and cellular mRNAs in infected cells. We will discuss why this might be advantageous for the virus.

MATERIALS AND METHODS

Purification and cleavage of the GST-NS1 fusion protein. The NS1 gene was inserted into the unique *Bam*HI site of the pGEX-3X (Pharmacia) expression vector, and the resulting vector was transformed into *Escherichia coli* JM101. The transformed cells were grown to an optical density at 600 nm of 0.8, isopropyl-β-D-thiogalactopyranoside (0.2 mM) was added for 2 h, and the cells were then harvested. The cells were suspended in phosphate-buffered saline (PBS) containing 1% Triton X-100 and 1 mM phenylmethylsulfonyl chloride and were disrupted by sonication. The resulting supernatant was applied to a glutathione Sepharose 4B affinity column (Pharmacia). After the column was washed with 30 ml of PBS, the glutathione *S*-transferase (GST)-NS1 fusion protein was eluted with 10 mM glutathione in 50 mM Tris-HCl, pH 8.0. Protein concentrations were determined by the Bio-Rad protein assay. The purity of the purified GST-NS1 fusion protein was established by sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by Coomassie blue staining. In most experiments, the NS1 protein was cleaved from the fusion protein with factor X (1:100 ratio with respect to the fusion protein) for 16 h at 4°C in 100 mM NaCl–50 mM Tris-HCl (pH 8.0)–1 mM $CaCl_2$. The completeness of cleavage was established by gel electrophoresis.

RNA binding assays. Full-length NS2 and M2 cDNAs were each inserted into the *Bam*HI site of pGEM1, or a 150-nucleotide poly(A) sequence containing *Eco*RI linkers was inserted into the *Eco*RI site of pGEM1. Labeled ([α-^{32}P]UTP or [α-^{32}P]ATP) or unlabeled transcripts were synthesized by using T7 or SP6 polymerase. Transcripts were purified by electrophoresis on 5% denaturing gels. The RNA binding assay was similar to that described by Heaphy et al. (9). The indicated amount of purified GST-NS1 fusion protein or nonfusion NS1 protein was incubated with a labeled RNA (10,000 cpm, approximately 0.5 ng) in an RNA binding buffer (43 mM Tris-HCl [pH 8], 50 mM KCl, 20 μg of *E. coli* transfer RNA, and 1 U of RNasin) in a final volume of 20 μl on ice for 20 min. Unlabeled competitor RNA was added during this incubation, where indicated. The protein-RNA complexes were separated from unbound RNA by electrophoresis on 4% nondenaturing polyacrylamide gels using 43 mM Tris-HCl (pH 8)–50 mM KCl as running buffer. After a 1- to 2-h prerun at 4°C, the gels were run at a 20-mA constant current for 24 h at 4°C with buffer recirculation. The gels were dried and visualized by autoradiography. The relative amounts of bound and unbound RNA were determined with a densitometer.

Transfection experiments. The NS1 protein was expressed by using an NS1 gene containing a 3′ splice site mutation

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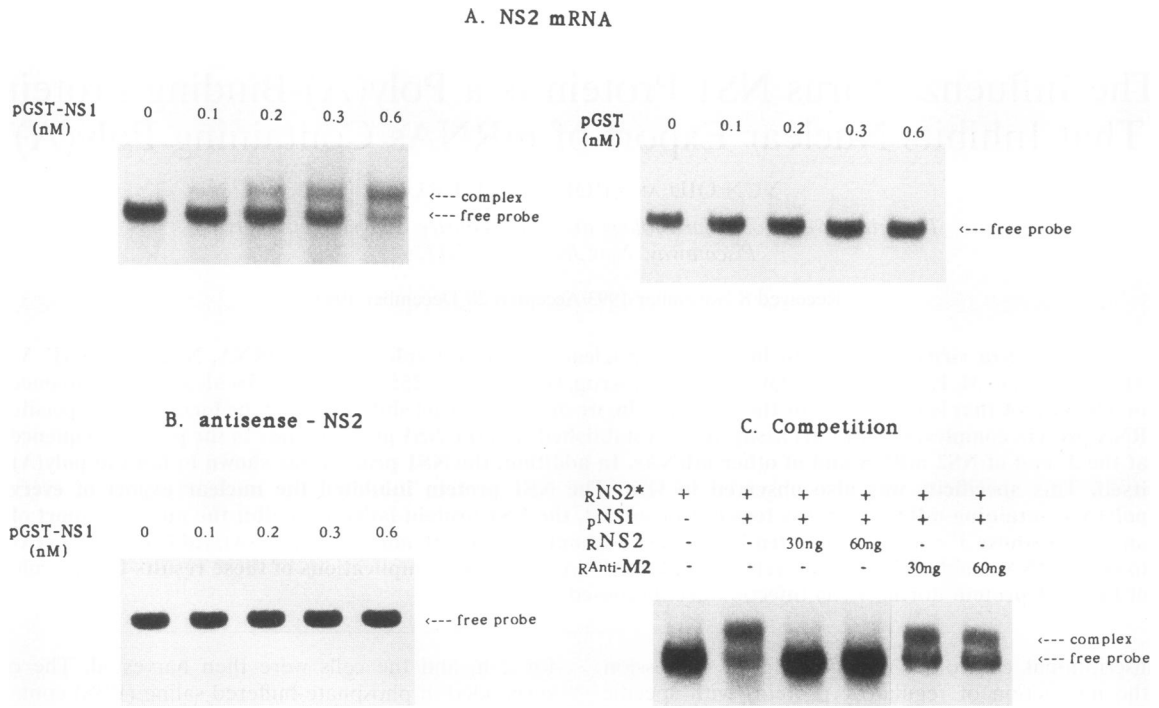

FIG. 1. The specific binding of the GST-NS1 fusion protein to NS2 mRNA assayed by gel shift. (A) A constant level (10,000 cpm, about 0.5 ng) of ^{32}P-labeled NS2 mRNA was incubated with an increasing amount of purified GST-NS1 fusion protein (left panel) or GST protein alone (right panel). Binding of this RNA to GST-NS1 resulted in a more slowly migrating RNA-protein complex upon electrophoresis on a 4% nondenaturing polyacrylamide gel. (B) The same amount of a ^{32}P-labeled antisense NS2 transcript was incubated with an increasing amount of the GST-NS1 fusion protein. (C) Competition experiments. Where indicated, unlabeled NS2 mRNA or unlabeled antisense M2 transcript was added to the binding reaction mixture at the levels shown.

(NS3ss); the control plasmid [NS3ss(dm)] contained two amber mutations in the NS1 protein reading frame (2). These two genes and the NS2, M2, PB1, and spliced β-globin minigene (cGL) were inserted into the *Bam*HI site of the PBC12 vector. All the mouse histone H4 constructs were in the pSP65 cytomegalovirus vector (8). H4-wt-proc contains the histone H4 coding region and the wild-type 3′ processing sequences including the 3′-proximal stem-loop structure. H4-mut-poly A contains two point mutations in the 3′-proximal stem-loop followed by a simian virus 40 polyadenylation signal. Transfection, cell fractionation, and RNA analysis were carried out as described by Qian et al. (21). Briefly, transfected cells at 40 h posttransfection were fractionated into nucleus and cytoplasm. The purity of the nuclear and cytoplasmic fractions was monitored with rRNA markers. Cell-equivalent amounts of nuclear and cytoplasmic RNAs were assayed for the amounts of specific RNAs by using S1 nuclease protection. Except for histone H4 mRNAs, specific 5′-end-labeled, single-stranded DNA probes were used as described by Alonso-Caplen et al. (2). For the histone H4 mRNAs, the 3′-end-labeled double-stranded DNA probe described by Eckner et al. (8) was used. After S1 nuclease digestion, the protected fragment(s) was resolved by gel electrophoresis. The relative amounts of a specific mRNA in the nucleus and cytoplasm were determined with a densitometer.

RESULTS

Specific RNA binding assay for the influenza virus NS1 protein. To determine the RNA binding specificity of the NS1 protein, we developed a gel shift assay. Purified NS1 protein was mixed with its target RNA, NS2 mRNA, at 4°C in the presence of a large amount of nonspecific competitor tRNA, and the mixture was subjected to gel electrophoresis under nondenaturing conditions to resolve the NS1 protein-NS2 mRNA complexes from unbound NS2 mRNA. In initial experiments we used a purified GST-NS1 fusion protein. As shown in Fig. 1A, increasing amounts of the GST-NS1 protein shifted increasing amounts of NS2 mRNA into a protein-RNA complex. The GST protein alone did not form a complex with NS2 mRNA, indicating that the NS1 protein part of the GST-NS1 fusion protein was responsible for the binding to NS2 mRNA.

The specificity of this binding assay was established in two ways. First, the antisense transcript of NS2 mRNA failed to form an RNA-protein complex with the GST-NS1 fusion protein (Fig. 1B). In addition, in competition experiments unlabeled NS2 mRNA eliminated complex formation between the NS1 fusion protein and labeled NS2 mRNA, whereas another unlabeled RNA, the antisense transcript of the viral M2 mRNA, did not inhibit complex formation (Fig. 1C). These results demonstrated that the GST-NS1 fusion protein binds to a sequence in NS2 mRNA that is not present in the antisense transcripts of the NS2 and M2 mRNAs.

In subsequent experiments the NS1 protein was cleaved from the GST-NS1 fusion protein. The nonfusion NS1 protein also formed a complex with NS2 mRNA but not with antisense NS2 mRNA (Fig. 2A and B). Figure 2C shows the comparison of the nonfusion NS1 protein and the GST-NS1 fusion protein with respect to binding efficiencies at various protein concentrations. At lower protein concentrations (0.3 nM or lower), the two proteins bound NS2 mRNA with similar efficiencies.

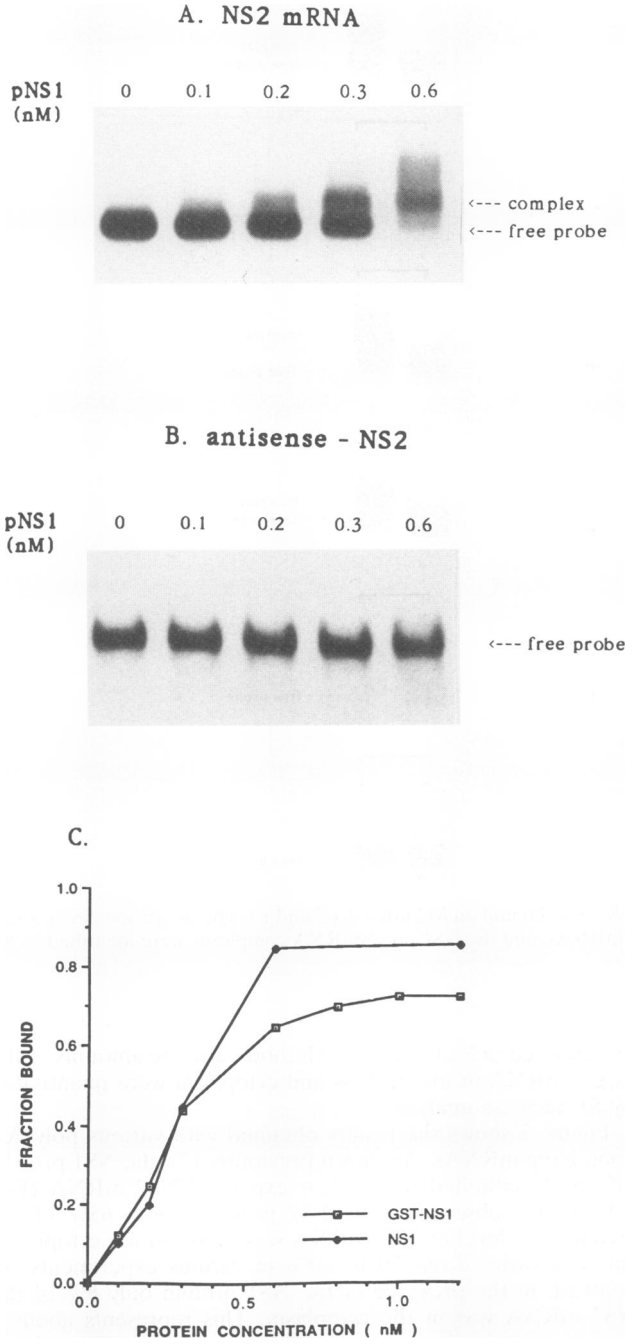

FIG. 2. Specific binding of the nonfusion NS1 protein to NS2 mRNA. (A) A constant level (10,000 cpm) of ^{32}P-labeled NS2 mRNA was incubated with an increasing amount of the nonfusion NS1 protein, and the NS1 protein-RNA complexes were separated from unbound mRNA by gel electrophoresis. (B) The same amount of a ^{32}P-labeled antisense NS2 transcript was incubated with an increasing amount of the nonfusion NS1 protein. (C) Fraction of the ^{32}P-labeled NS2 mRNA that is bound to either the nonfusion NS1 protein or the GST-NS1 fusion protein at increasing concentrations of either protein. The data are from several gel shift experiments, including those of Fig. 1A and 2A.

At higher concentrations, however, the nonfusion NS1 protein bound NS2 mRNA with higher efficiency than the GST-NS1 fusion protein. From these data, we calculated the K_d for the nonfusion NS1 protein to be approximately 0.4 nM.

Identification of poly(A) as the binding site of the NS1 protein. To identify the binding site of the NS1 protein in the NS2 mRNA sequence, we carried out 5′ and 3′ deletions of the NS2 mRNA sequence (Fig. 3). The first deletions that were made identified the binding site. When the 20 A residues at the 3′ end of NS2 mRNA were removed, all complex formation with the nonfusion NS1 protein was eliminated. In contrast, deletion of either 16 nucleotides from the 5′ end of NS2 mRNA or 20 nucleotides elsewhere in NS2 mRNA did not affect complex formation. This indicated that the NS1 protein was binding to the 20-nucleotide poly(A) sequence at the 3′ end of NS2 mRNA. The same result was obtained with another viral mRNA, M2 mRNA. The NS1 protein formed a complex with the M2 mRNA containing 19 3′-terminal A residues, and deletion of these 3′-terminal A residues eliminated binding. It should be noted that antisense NS2 and M2 mRNA transcripts, which either did not bind to the NS1 protein or did not compete with NS2 mRNA for binding, do not contain a poly(A) stretch. Rather, these RNAs contain a 19- or 20-nucleotide poly(U) stretch, indicating that the NS1 protein does not bind to poly(U).

To verify this binding specificity, poly(A) itself was used as the target RNA. This poly(A) was 150 nucleotides in length and, hence, 7.5 times longer than the poly(A) sequences in the NS2 and M2 mRNAs used in the experiments of Fig. 1 through 3. Because the NS1 protein binds to the 19- or 20-nucleotide poly(A) sequence in NS2 and M2 mRNA, the 150-nucleotide poly(A) molecule would be expected to bind more than one NS1 protein molecule. As shown in Fig. 4A, the nonfusion NS1 protein efficiently formed complexes with poly(A) itself. At the concentration (0.2 nM) of the NS1 protein at which binding first occurred, the resulting complexes had gel mobilities slightly faster than that of the fully formed NS1 protein-poly(A) complexes. We presume that these species represent poly(A) molecules which contained fewer NS1 protein molecules than the fully formed complexes. At the next higher concentration (0.4 nM) of the NS1 protein, all the complexes with the NS1 protein were fully formed. Only a small amount of unbound poly(A) remained. The amount of poly(A) that remained unbound at high concentrations of the NS1 protein varied between experiments (see below). Assuming that multiple NS1 protein molecules associated with the fully formed complexes, the results suggest that there is moderate cooperativity in the binding of the NS1 protein to the 150-nucleotide poly(A) molecules, as defined with other nucleic acid binding proteins (14). The presence of only a small amount of partially complexed NS1 protein-poly(A) intermediate (at 0.2 nM of the NS1 protein) is characteristic of moderate cooperativity. If there were no cooperativity, there would be a succession of discrete intermediate species as the concentration of the NS1 protein was increased.

As other poly(A)-binding proteins have been shown to bind to other homopolymers, e.g., poly(U) and poly(G) (3, 5, 18, 25, 26), we determined whether other homopolymers would compete with labeled poly(A) for binding to the NS1 protein. Because it has already been established that the NS1 protein does not bind to a 19- or 20-nucleotide poly(U) stretch and because poly(U) would anneal to the labeled poly(A) in competition experiments, we tested only poly(C) and poly(G). The control competition was with unlabeled poly(A). With 60 ng of unlabeled poly(A), almost all of the labeled poly(A) was displaced from a complex with the NS1 protein, and this

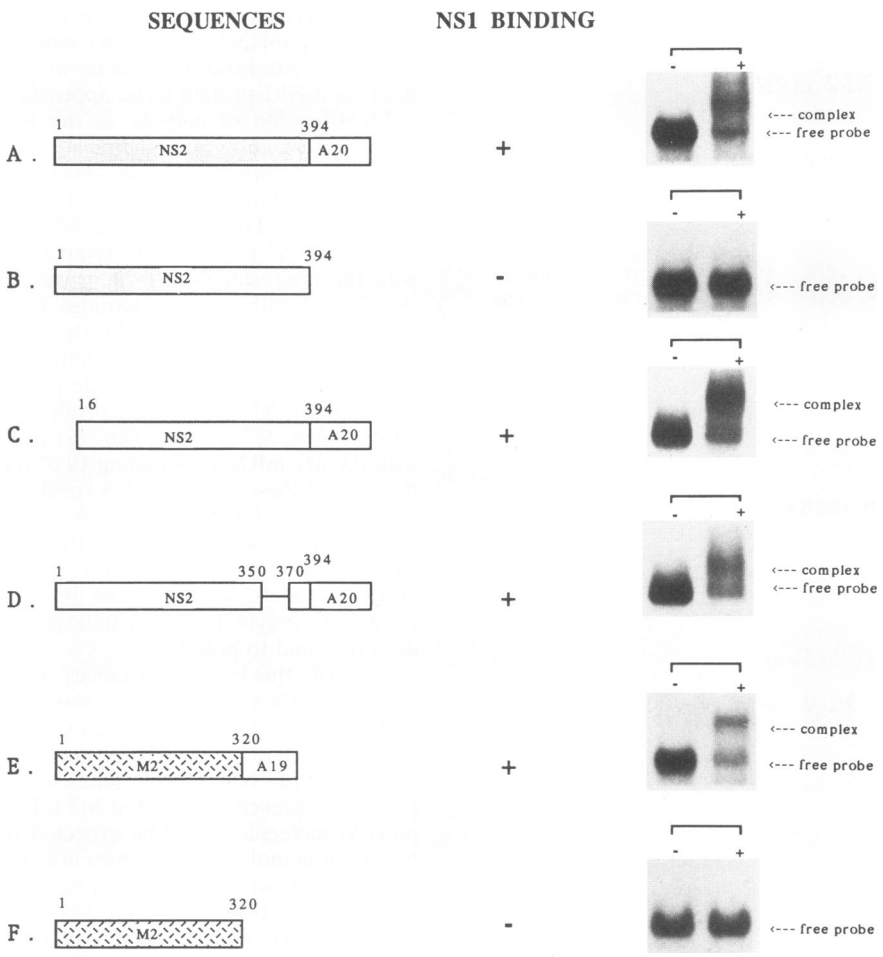

FIG. 3. Identification of the binding site of the NS1 protein on NS2 mRNA (A to D) and on M2 mRNA (E and F). The nonfusion NS1 protein (0.4 nM) was incubated with the indicated full-length or deleted NS2 or M2 mRNAs, and the NS1 protein-RNA complexes were identified by gel electrophoresis.

displacement was complete with 120 ng of unlabeled poly(A) (Fig. 4B). In contrast, unlabeled poly(C), even at 120 ng, did not compete with labeled poly(A) for binding to the NS1 protein (Fig. 4C). However, unlabeled poly(G) displaced labeled poly(A) from the NS1 protein complex even more efficiently than unlabeled poly(A) itself (Fig. 4D). The lowest level of poly(G) tested, 30 ng, already displaced all the labeled poly(A). This indicates that the NS1 protein recognizes poly(G) as well as poly(A).

NS1 protein exhibits specificity for poly(A) in vivo. The finding that the NS1 protein recognizes and binds to poly(A) was surprising for several reasons, including the following: the RNA-binding domain of the NS1 protein does not contain the consensus sequences of other poly(A)-binding proteins (3, 5, 21, 25, 26), and our own earlier data indicated that the NS1 protein-mediated inhibition of nuclear mRNA export was apparently specific for the viral NS2 mRNA (2). Accordingly, we first reexamined the ability of the NS1 protein to inhibit the nuclear export of various poly(A)-containing mRNAs, both viral and cellular. 293 cells were cotransfected with two plasmids: one encoding the target mRNA and one encoding the NS1 protein via an unspliceable NS1 mRNA, i.e., an NS1 mRNA containing a 3′ splice site mutation. At 40 h posttransfection the cells were fractionated into nucleus and cytoplasm as described in Materials and Methods, and the amounts of the target mRNA in the nucleus and cytoplasm were quantitated by S1 nuclease analysis.

Figure 5 shows the results obtained with various poly(A)-containing mRNAs. As shown previously (2), the NS1 protein effectively inhibited the nuclear export of NS2 mRNA (Fig. 5A). In the absence of the NS1 protein, about 65% of the steady-state level of NS2 mRNA was found in the cytoplasm; this has varied from 50 to 80% in various experiments. In contrast, in the presence of the NS1 protein only 8% of the NS2 mRNA was in the cytoplasm. This represents about a 22-fold shift in the nucleus/cytoplasm ratio of NS2 mRNA caused by the NS1 protein. The NS1 protein had the same effect on other viral mRNAs, inhibiting the nuclear export of M2 mRNA (Fig. 5B), PB1 mRNA (Fig. 5C), and the hemagglutinin and nucleocapsid protein mRNAs (data not shown). In addition, the NS1 protein inhibited the nuclear export of spliced globin mRNA (Fig. 5D) and the spliced Tat mRNA of human immunodeficiency virus type 1 (data not shown). In fact, the NS1 protein inhibited the nuclear export of every poly(A)-containing mRNA that we tested, thereby refuting our earlier conclusion (2). It should be noted that in the experiments in Fig. 6 the total amount of each of the mRNAs is somewhat greater in the presence than in the absence of the

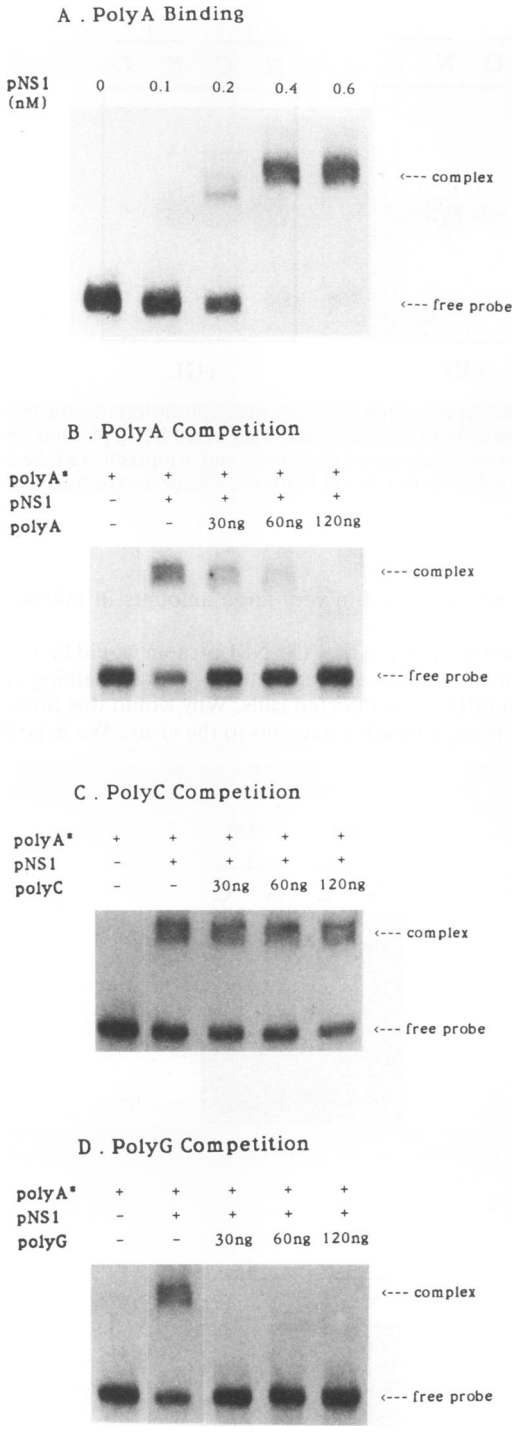

FIG. 4. The NS1 protein binds to poly(A) itself, and this binding is displaced by poly(G). (A) A constant level (10,000 cpm) of the ^{32}P-labeled 150-nucleotide poly(A) was incubated with an increasing amount of the nonfusion NS1 protein, and the protein-RNA complexes were separated from unbound RNA by gel electrophoresis. (B to D) Experiments with the indicated unlabeled competitors.

NS1 protein. This is due at least in part to the stabilization of mRNA molecules in both the nucleus and the cytoplasm by the NS1 protein, as shown previously (2).

All of the mRNAs so far tested contain 3′-terminal poly(A) sequences. To establish that the NS1 protein-mediated inhibition of nuclear export of mRNA required poly(A), it was necessary to utilize a mRNA that lacked poly(A). We used a histone mRNA whose 3′ end is generated by cleavage without the subsequent addition of poly(A) (8). As shown in Fig. 6A, in the presence of the NS1 protein the vast majority of the histone mRNA was in the cytoplasm (nucleus/cytoplasm ratio, 0.2), indicating that the NS1 protein did not inhibit the nuclear export of this mRNA. As in previous experiments (see above), the NS1 protein caused some stabilization of this mRNA in both the nucleus and the cytoplasm. In contrast to the histone mRNA, a poly(A)-containing mRNA in the same cells, NS1 mRNA containing a 3′ splice site mutation, was retained in the nucleus by the NS1 protein (nucleus/cytoplasm ratio, 10).

Poly(A) was added to the histone mRNA to determine whether such an addition would render the nuclear export of the histone mRNA susceptible to inhibition by the NS1 protein. A 3′ polyadenylation signal was substituted for the histone 3′ cleavage process. As shown in Fig. 6B, the NS1 protein inhibited the nuclear export of the resulting poly(A)-containing mRNAs, as evidenced by the dramatic shift (20-fold) in the nucleus/cytoplasm ratio of these mRNAs caused by the NS1 protein. The nuclear export of the poly(A)-containing NS1 mRNA (with a 3′ splice site mutation) in the same cells was similarly inhibited. Thus, the addition of a 3′ poly(A) sequence rendered the nuclear export of the histone mRNA susceptible to inhibition by the NS1 protein.

DISCUSSION

We have shown that the influenza virus NS1 protein is a poly(A)-binding protein. It specifically binds to poly(A) sequences in vitro. This specificity is also exhibited in vivo, as the ability of the NS1 protein to inhibit the nuclear export of an mRNA is dependent on the mRNA containing a poly(A) sequence.

The NS1 protein is thus another member of the group of proteins that bind to poly(A). Several cellular poly(A)-binding proteins have been identified. A 70- to 72-Da protein, PAB1, which is the major poly(A)-binding protein, is bound to the 3′ poly(A) sequences of mRNAs in the cytoplasm (1, 4, 5). This protein binds to poly(G) and poly(U) as well as poly(A) (5). A second protein, PABII, facilitates the elongation of oligo(A) tails during the generation of the 3′ poly(A) ends of mRNAs (25, 26). This protein binds to poly(G) as well as poly(A). A third poly(A)-binding protein, PUB1, is found in yeast cells and actually has a strong preference for binding to poly(U) (3, 18). Like these cellular poly(A)-binding proteins, the influenza virus NS1 protein also recognizes another homopolymer in addition to poly(A), in this case poly(G). However, as shown by Qian et al. (21), the RNA-binding domain of the NS1 protein does not contain the RNA-binding consensus sequences found in the cellular PABI, PABII, and PUB1 proteins (3, 5, 25, 26).

One cellular poly(A)-binding protein, and probably more, is located in the nucleus. The PABII protein, which participates in the nuclear activity of polyadenylation, is found in the nucleus (26), and the PUB1 protein has been found in both the nucleus and the cytoplasm (3, 18). It is not known whether cellular poly(A)-binding proteins in the nucleus participate in functions in addition to polyadenylation, and experiments addressing a possible role for poly(A) in the nuclear export of

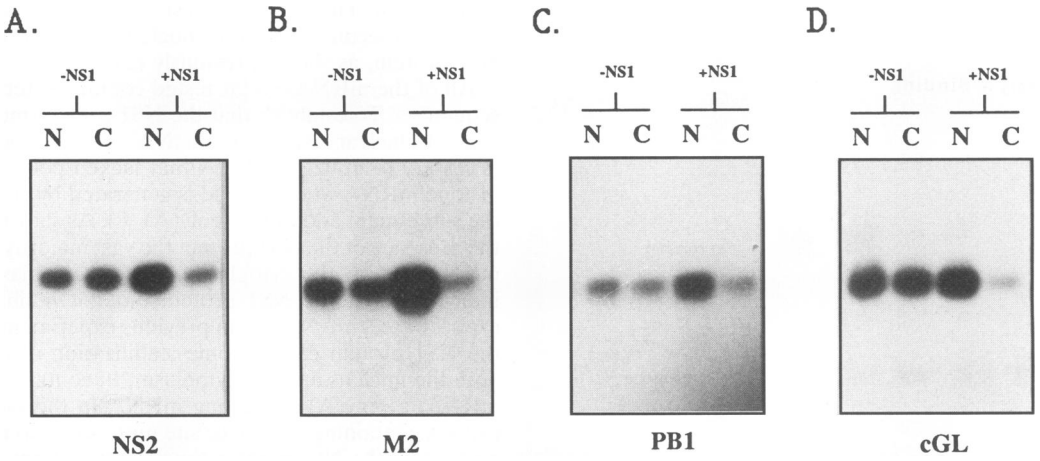

FIG. 5. The NS1 protein inhibits the nuclear export of all poly(A)-containing mRNAs examined. 293 cells were cotransfected with 15 μg of a PBC12 plasmid either encoding the NS1 protein (NS3ss) or not encoding the NS1 protein [NS3ss(dm)] and 5 μg of a PBC12 plasmid encoding the indicated target poly(A)-containing mRNA. At 40 h after transfection, the cells were fractionated into nuclei and cytoplasm, and the nuclear (N) and cytoplasmic (C) RNAs were extracted and subjected to S1 analysis as described in Materials and Methods. The protected fragments were resolved by gel electrophoresis.

mRNAs have not been conclusive (discussed in reference 22). The influenza virus NS1 protein would have to compete with the cellular poly(A)-binding proteins in the nuclei of infected cells. In fact, the NS1 protein would be expected to have a competitive advantage: it has a K_d similar to, or lower than, the K_d of cellular poly(A)-binding proteins (23, 26), and the NS1 protein is synthesized in very large amounts in infected cells (13).

Our results indicate that the NS1 protein would be expected to inhibit the nuclear export of all poly(A)-containing cellular and viral mRNAs in infected cells. Why would this function of the NS1 protein be advantageous to the virus? We hypothesize

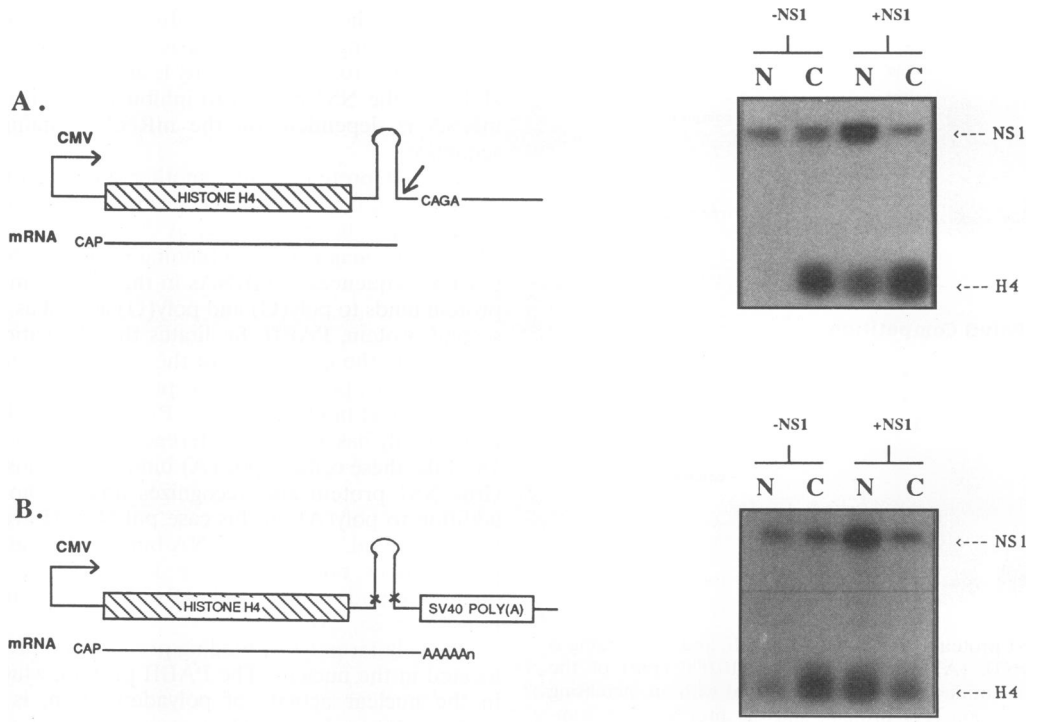

FIG. 6. Poly(A) dependence of the inhibition of nuclear export of an mRNA by the NS1 protein. 293 cells were cotransfected with 15 μg of the PBC12-NS3ss plasmid (+NS1 protein) or the PBC12-NS3ss(dm) plasmid (−NS1 protein) and 5 μg of the pSp65CMV plasmid encoding the indicated target histone mRNA. The nuclear (N) and cytoplasmic (C) RNAs were subjected to S1 analysis for both the target histone mRNA and the NS1 mRNA encoded by the PBC12 plasmids. The S1-protected fragments were resolved by gel electrophoresis. CMV, cytomegalovirus; SV40, simian virus 40.

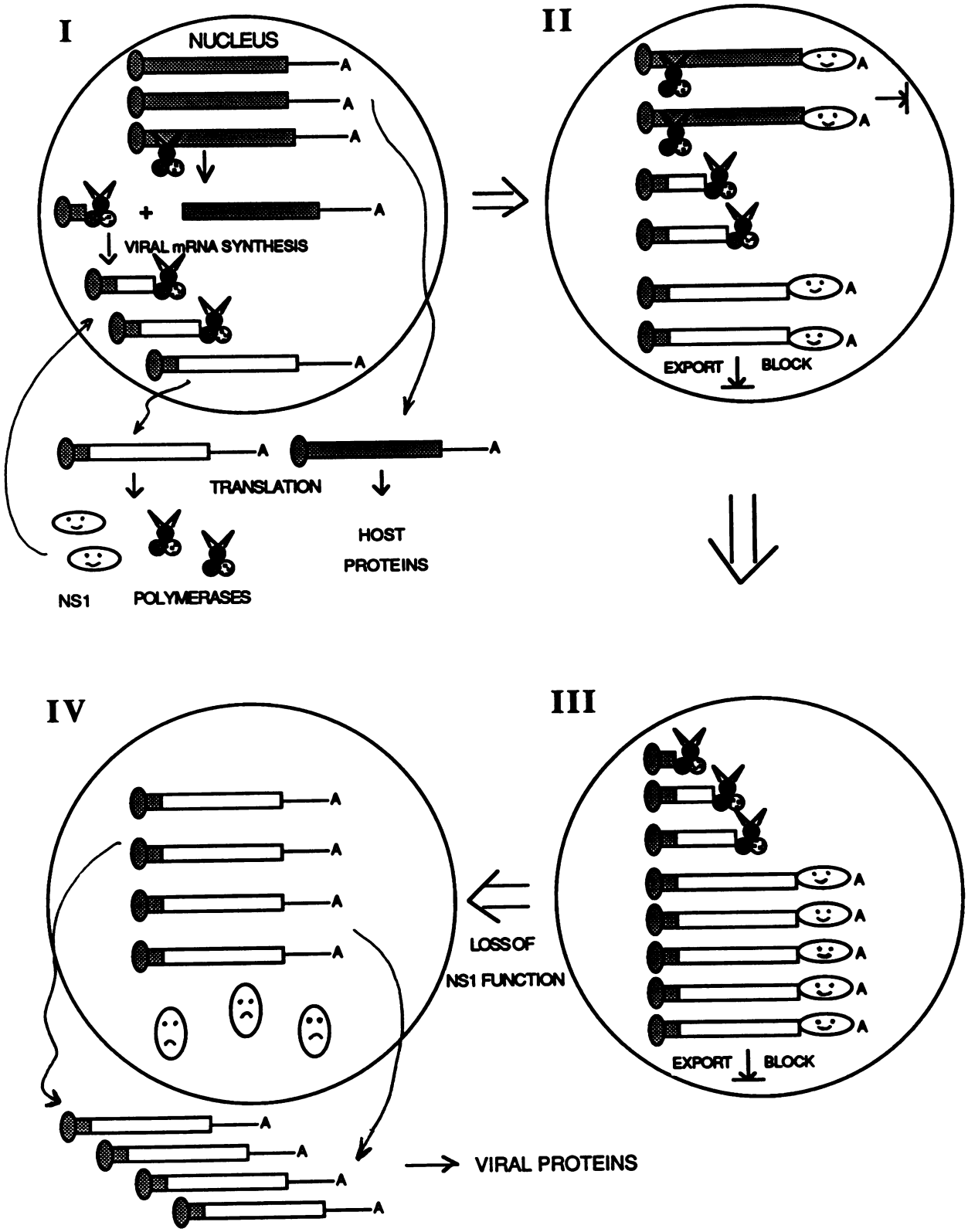

FIG. 7. Postulated function of the NS1 protein during influenza virus infection.

that this function is needed at appropriate times of infection to make the cellular mRNAs accessible to cleavage by the viral polymerase for the generation of the capped primers, which are needed for viral mRNA synthesis (13, 19) (Fig. 7). At the beginning of infection, cellular mRNAs would freely exit from the nucleus. Utilizing primers generated from cellular mRNAs before they exit from the nucleus, the viral polymerase would synthesize mRNAs, particularly those encoding the NS1 protein and the constituents of the polymerase-nucleocapsid complex. Indeed, it has been shown that the NS1 protein and the nucleocapsid protein are preferentially made at early times after infection (13). When enough NS1 protein has accumulated in the nucleus, it would block the transport of both cellular and viral mRNAs. As a consequence, the cellular mRNAs would be sequestered or trapped in the nucleus, thereby making them accessible to cleavage by the viral polymerase to generate capped primers. Viral mRNA synthesis would then occur with the required efficiency, and all cellular mRNAs and their precursors, which would have lost their protective 5′ capped ends, would be degraded in the nucleus. In fact, the degradation of cellular mRNAs in the nuclei of infected cells has been documented (11, 13). The nuclei of infected cells would become packed with only viral mRNAs. In order for these viral mRNAs to exit from the nucleus, the NS1 protein would have to lose function at this time of infection. Either the NS1 protein might lose its ability to bind to the poly(A) at the 3′ ends of the mRNAs (as shown in Fig. 7) or the second functional domain of the NS1 protein (21) might be inactivated. Such a loss of function could result from a posttranscriptional modification of the NS1 protein. The NS1 protein contains such modifications: it is a phosphoprotein and may also contain another modification, because two-dimensional gel electrophoresis indicates the presence of two nonphosphorylated forms of the NS1 protein (20). Support for this part of the hypothesis comes from the results of experiments using a protein kinase inhibitor (H7) or a methyltransferase inhibitor (24). Either inhibitor caused viral mRNAs to accumulate in the nucleus, suggesting that blocking posttranscriptional modification of the NS1 protein causes it to retain its function in inhibiting the nuclear export of mRNAs. However, this is not definitive evidence, because these inhibitors could also affect other proteins and functions in infected cells. We are currently testing this hypothesis for the role of the NS1 protein in infected cells.

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