JOURNAL OF VIROLOGY, June 1993, p. 3586–3595
0022-538X/93/063586-10$02.00/0

Vol. 67, No. 6

RNA-Binding Properties of a Translational Activator, the Adenovirus L4 100-Kilodalton Protein

DEBORAH RILEY AND S. J. FLINT*

Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544

Received 24 November 1992/Accepted 15 March 1993

The adenovirus L4 100-kDa nonstructural protein (100K protein) is required for efficient initiation of translation of viral late mRNA species during the late mRNA species during the late phase of infection (B. W. Hayes, G. C. Telling, M. M. Myat, J. F. Williams, and S. J. Flint, J. Virol. 64:2732–2742, 1990). The RNA-binding properties of this protein were analyzed in an immunoprecipitation assay with the 100K-specific monoclonal antibody 2100K-1 (C. L. Cepko and P. A. Sharp, Virology 129:137–154, 1983). Coprecipitation of the 100K protein and ^{3}H-infected cell RNA was demonstrated. The RNA-binding activity of the 100K protein was inhibited by single-stranded DNA but not by double-stranded DNA, double-stranded RNA, or tRNA. Competition assays were used to investigate the specificity with which the 100K protein binds to RNA in vitro. Although the protein exhibited a strong preference for the ribohomopolymer poly(U) or poly(G), no specific binding to viral mRNA species could be detected; uninfected or adenovirus type 5-infected HeLa cell poly(A)-containing and poly(A)-lacking RNAs were all effective inhibitors of binding of the protein to viral late mRNA. Similar results were obtained when the binding of the 100K protein to a single, in vitro-synthesized L2 mRNA was assessed. The poly(U)-binding activity of the 100K protein was used to compare the RNA-binding properties of the 100K protein prepared from cells infected by adenovirus type 5 and the H5ts1 mutant (B. W. Hayes, G. C. Telling, M. M. Myat, J. F. Williams, and S. J. Flint, J. Virol. 64:2732–2742, 1990). A temperature-dependent decrease in H5ts1 100K protein binding was observed, correlating with the impaired translational function of this protein in vivo. By contrast, wild-type 100K protein RNA binding was unaffected by temperature. These data suggest that the 100K protein acts to increase the translational efficiency of viral late mRNA species by a mechanism that involves binding to RNA.

Selective translation of viral late mRNAs occurs at late times after adenovirus infection of permissive cells (for reviews, see references 23, 37, and 50). The mechanisms that lead to efficient translation of viral mRNA but inhibition of cellular protein synthesis have not been fully elucidated. However, suppression of cellular translation requires progression into the late phase (26), and neither the stability nor the in vitro translatability of cellular mRNAs is decreased (6, 56). Three virus-encoded functions, VA1 RNA, the tripartite leader sequence, and the L4 100-kDa nonstructural protein (100K protein), have been shown to increase translational efficiency during the late phase of infection. These viral activators of translation may also be involved in translational discrimination between viral late and cellular mRNAs.

Adenovirus VA1 RNA is a small RNA polymerase III transcript required for the continued initiation of translation during the late phase (37, 39, 49, 56). Following infection by a variety of viruses, an interferon-induced kinase, termed DAI or EIF-2α kinase, inhibits translation by phosphorylating the α subunit of the initiation factor EIF-2 (45). The major role of VA1 RNA is believed to be to counter this host antiviral response, by preventing activation of EIF-2α kinase. In cells infected by *dl*331, an adenovirus mutant that fails to synthesize VA1 RNA, both cellular and viral protein synthesis is drastically reduced because of a lack of functional EIF-2 (49). It has been proposed that VA1 RNA functions by binding to EIF-2α kinase, thus blocking its activation and preserving the function of EIF-2 (32). VA1 RNA may not be essential for translational discrimination between viral and cellular mRNAs, for in many adenovirus-infected cell lines, host mRNA translation is inhibited without measurable activation of EIF-2α kinase. Furthermore, the drug 2-aminopurine prevents inhibition of host mRNA translation without altering EIF-2α kinase activity (30).

The tripartite leader is a 202-nucleotide, 5′ untranslated sequence common to most viral late mRNAs, those transcribed from the major late promoter. The leader increases translation of heterologous mRNAs during the late, but not the early, phase of infection and does not increase translational efficiency when placed at the 3′ end of mRNA, suggesting a role in initiation (2, 11, 33, 35). It has been proposed that the tripartite leader might reduce or eliminate the requirement for the cap-binding EIF-4F complex (20). Since adenovirus infection results in underphosphorylation of cap-binding protein EIF-4E (31) and inactivation of the EIF-4F complex, such independence from EIF-4F activity could be important for discrimination of adenovirus late mRNAs from cellular mRNAs.

We have shown that the adenovirus L4 100K protein is required for efficient translation of late but not early viral mRNA species (29). This protein, which is not found in mature virions, is synthesized in large quantities from the beginning of the late phase of infection (10) and is a major viral phosphoprotein (5, 24, 47). The mechanism of action of the 100K protein is presently unknown. However, the protein binds to RNA in vivo (1) and appears to be associated with polyribosomal viral RNA or ribosomes (34, 52, 55, 58). Therefore, we have investigated the specificity with which the 100K protein binds to RNA and compared in vitro RNA-binding activity with in vivo function, using a temperature-sensitive adenovirus L4 mutant, H5ts1 (60).

* Corresponding author.

MATERIALS AND METHODS

Cells and viruses. Adenovirus type 5 (Ad5) was grown in HeLa suspension cells at 37°C. These cells were maintained in suspension minimal essential medium supplemented with 5% calf serum. The Ad5 temperature-sensitive mutant H5ts1 was grown in monolayer cultures of the Ad5-transformed human embryonic kidney 293 cell line (28) at 33°C. 293 cells were maintained in Dulbecco modified Eagle medium supplemented with 5% fetal calf serum, 5% bovine calf serum, and 1% glutamine. The infectivities of wild-type and mutant virus stocks were measured by plaque assay on 293 cells.

Protein labeling. Proteins were labeled for 1 h at 33°C with 100 μCi of ^{35}S-protein-labeling mix, per 90-mm plate; the mix contains approximately 77% [^{35}S]methionine (specific activity, >1,000 Ci/mmol; New England Nuclear). The labeling mix was added to 2 ml of methionine-free Dulbecco modified Eagle medium. At the end of the labeling period, cells were harvested and cytoplasmic extracts were prepared as described in the following section.

Preparation of cytoplasmic extracts. All procedures were performed at 4°C. Medium was removed from plates, and cells were scraped into phosphate-buffered saline (PBS), washed twice in 10 volumes of PBS, and resuspended in homogenization buffer (50 mM Tris [pH 7.5], 1 mM EDTA, 5 mM dithiothreitol, 10% glycerol). Following 10 strokes with a Dounce homogenizer, the disrupted cells were centrifuged at 2,500 × *g* for 20 min to remove nuclei. The supernatant or cytoplasmic fraction was divided into small portions and frozen at −80°C.

Preparation and analysis of RNA. Cytoplasmic RNA was prepared as described by Sambrook et al. (48) either from uninfected 293 cells or cells harvested during the late phase of adenovirus infection (18 to 22 h). Labeled RNA was prepared by incubating the cells with 1 mCi of [^{3}H]uridine per 90-mm plate for 4 h at 37°C in unsupplemented Dulbecco modified Eagle medium. Briefly, medium was removed and cells were scraped into ice-cold PBS, washed twice in 10 volumes of PBS, and resuspended in 200 μl of RNA extraction buffer (0.14 M NaCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl, 1 mM dithiothreitol, 0.5% Nonidet P-40 [pH 8.6]) per 90-mm plate. The suspension was vortexed and incubated at 4°C for 5 min. Following centrifugation at 12,000 × *g* for 90 s at 4°C, the supernatant was taken and an equal volume of proteinase digestion buffer (0.2 M Tris-HCl [pH 8.0], 25 mM EDTA, 0.3 M NaCl, 2% sodium dodecyl sulfate [SDS]) was added. Proteinase K was added to a final concentration of 20 μg/ml, and the mixture was incubated for 30 min at 37°C. An RNA pellet was recovered following phenol-chloroform extraction, isopropanol precipitation, and a 70% ethanol wash. Poly(A)$^+$ and poly(A)$^-$ fractions were prepared with Hybond mAP messenger affinity paper (Amersham) according to the manufacturer's instructions. All RNA was stored at −80°C under ethanol. To ensure that RNA preparations were intact, portions were glyoxylated and analyzed by electrophoresis in 1% agarose gels containing 10 mM phosphate buffer (pH 6.0). RNA was either stained with ethidium bromide or, in the case of ^{3}H-labeled RNA, visualized by fluorography with En^{3}Hance (NEN). Ribohomopolymers, tRNA, double-stranded RNA [poly(rA-rU) · poly(rA-rU)], and DNA [poly(dI-dC)] were purchased from Pharmacia.

Immunoprecipitation assays. Cytoplasmic extracts from infected cells were incubated with ^{3}H-infected cell RNA for 30 min at 22°C in RBB (RNA-binding buffer; 10 mM Tris-HCl [pH 7.5], 3 mM $MgCl_2$, 50 mM NaCl, 0.5% Nonidet P-40, 1 mM dithiothreitol). In preliminary studies, these conditions were found to be optimal for RNA-100K protein binding. For binding specificity studies, competitor RNAs were included in the binding reactions. The monoclonal antibody 2100K-1 (16) was purified from serum-depleted hybridoma cell culture medium. Following removal of the hybridoma cells by centrifugation at 1,500 × *g* for 20 min, the supernatant fraction was applied to a protein A-Sepharose column. The column was washed with PBS and antibody was eluted with 0.1 M glycine-HCl (pH 3). The pH of the eluate fractions was immediately adjusted with 1 M Tris (pH 7.5), and the concentration of immunoglobulin G was determined by the Bradford assay. The 2100K-1 antibody (10 μg per tube) was added to the binding reaction mixtures, and incubation continued for 1 h at 4°C. Protein A-Sepharose (50 μl of a 1:1 slurry in RBB) was then added, and incubation continued for 1 h, with vortexing every 10 min. Unbound RNA was removed by centrifugation at 12,000 × *g* for 30 s and aspiration of the supernatant. The Protein A-Sepharose pellet was washed three times with 1 ml of RBB. Bound RNA was extracted by heating the protein A-Sepharose pellets to 90°C for 5 min in 50 μl of 2% SDS–0.125 M Tris-HCl (pH 6.8). The protein A-Sepharose was removed by centrifugation at 12,000 × *g* for 1 min, and bound RNA in the supernatant fraction was quantitated by scintillation counting.

Poly(U)-Sepharose binding of the 100K protein. [^{35}S]methionine-labeled cytoplasmic proteins from uninfected or infected cells were incubated with poly(U)-Sepharose (Pharmacia) preequilibrated in RBB for 1 h with mixing every 10 min. Bound proteins were recovered by centrifugation at 12,000 × *g* for 1 min and washed three times with RBB. Proteins were extracted from the poly(U)-Sepharose pellets by being heated in 2× SDS sample buffer (2% SDS, 0.125 M Tris-HCl [pH 6.8], 100 mM dithiothreitol, 10% glycerol, 2% bromophenol blue). ^{35}S-labeled proteins were then separated by SDS-polyacrylamide gel electrophoresis (PAGE) and visualized by autoradiography.

RESULTS

Characterization of 100K protein-RNA binding by an immunoprecipitation assay. Previous studies have demonstrated that the 100K protein can be cross-linked to RNA upon exposure of adenovirus-infected cells to UV light (1). Although this assay demonstrated that the 100K protein and RNA interact in vivo (1), it is not well-suited for quantitative analysis; cross-linking efficiency may, for example, be determined as much by the chemistry of the light-activated reaction as by the affinity of protein for its RNA ligand. Therefore, we sought an in vitro assay system for the RNA-binding activity of the adenovirus L4 100K protein that would permit quantitative comparison of its binding to different types of RNA. In initial studies, the suitability of mobility shift gel assays with a viral late mRNA (synthetic L2 mRNA) as probe for the study of 100K protein binding was investigated. In these experiments, the binding of several cellular proteins to the RNA probe yielded bands with reduced mobility in nondenaturing polyacrylamide gels (data not shown). However, no difference between uninfected and infected cell extracts could be detected. It is possible that the binding of cellular proteins to L2 RNA obscured binding by the 100K protein. In other experiments, late proteins were labeled with [^{35}S]methionine, and binding to filter-immobilized RNA was analyzed. The 100K protein did not bind to RNA under these conditions, suggesting that structure is important for its interaction with RNA.

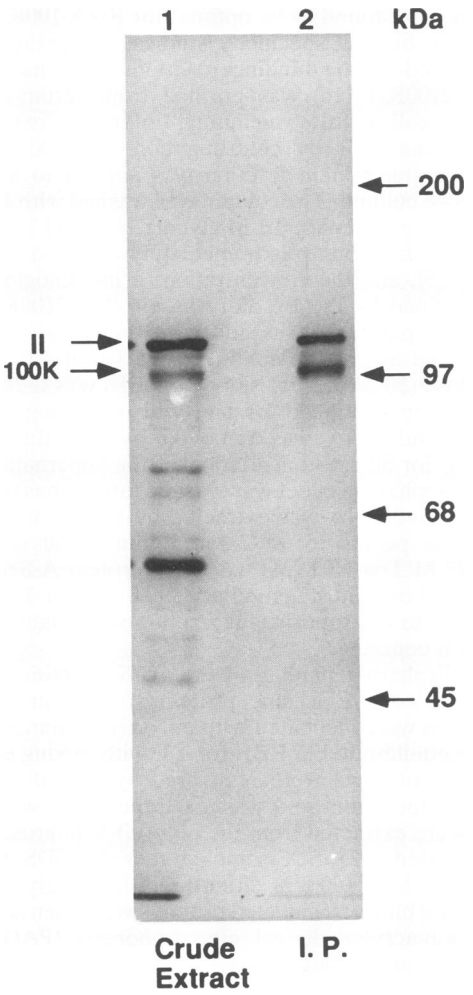

FIG. 1. Immunoprecipitation of cytoplasmic proteins with the 100K protein antibody 2100K-1. Proteins were labeled with [^{35}S]methionine late in infection, and immunoprecipitation was performed as described in Materials and Methods. Proteins were separated by SDS-PAGE and visualized by autoradiography. Lanes: 1, cytoplasmic extract; 2, immunoprecipitated proteins (I.P.).

To investigate the specificity with which the 100K protein binds to RNA, coprecipitation of radiolabeled RNA bound to the 100K protein was analyzed by an immunoprecipitation assay with the 2100K-1 monoclonal antibody (16). Such an immunoprecipitation assay permitted RNA bound to the 100K protein in solution to be analyzed and quantitated without complications introduced by the activities of other RNA-binding proteins present in infected cell extracts. It also permitted the use of a nonuniform RNA probe consisting of a mixture of RNA species. This was important, because the identities of any specific RNA sequences that might be bound by the 100K protein were not known. The assay was characterized by immunoprecipitating ^{35}S-labeled, infected cell cytoplasmic proteins under conditions identical to those used for subsequent RNA-binding analyses. In Fig. 1, cytoplasmic proteins are shown prior to (lane 1) and following (lane 2) immunoprecipitation with the monoclonal antibody 2100K-1, which recognizes the native 100K protein (16). The only proteins immunoprecipitated were 100K and late polypeptide II (hexon). It has been

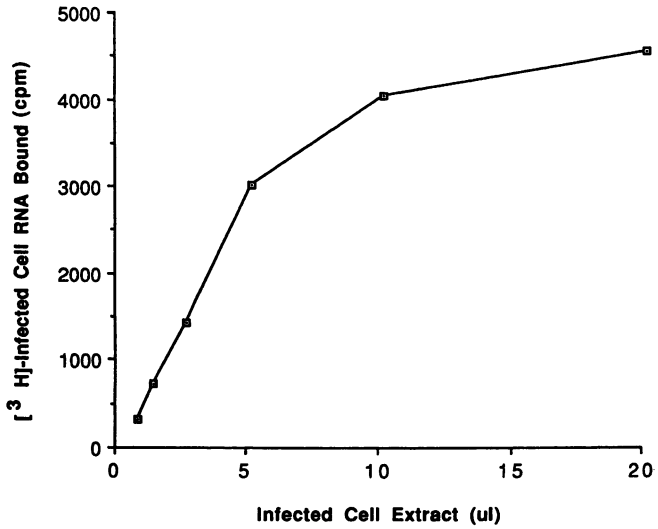

FIG. 2. Binding of the 100K protein to infected cell RNA in an immunoprecipitation assay. Increasing quantities of cytoplasmic extracts of Ad5-infected cells (1 μg/μl) were incubated with [^{3}H] uridine-labeled RNA (500,000 cpm). Immunoprecipitation of 100K protein RNA complexes was as described in Materials and Methods.

reported previously that the 100K protein forms a complex with polypeptide II that can be immunoprecipitated by the 2100K-1 antibody (16).

To examine binding of the 100K protein to RNA, cytoplasmic extracts prepared from unlabeled infected 293 cells were incubated with [^{3}H]uridine-labeled infected cell RNA and immunoprecipitated. RNA was recovered and quantitated by scintillation counting. When increasing amounts of cytoplasmic protein were added to a constant amount of RNA, the quantity of RNA bound increased linearly up to 50 μg of protein added. At higher protein concentrations, there was no further increase in bound RNA (Fig. 2). The maximum amount of RNA bound was between 0.7 and 1% of that added. In control experiments, uninfected extracts were substituted for infected cell extracts in the RNA-binding assay. Because these extracts did not contain any 100K protein, no RNA was recovered following immunoprecipitation with the 2100K-1 antibody (data not shown). Furthermore, when the E1A antibody M73 replaced the 2100K-1 antibody in RNA-binding assays with infected cell extracts, no bound RNA was recovered (data not shown). These results indicate that the RNA recovered following immunoprecipitation with antibody 2100K-1 was a result of 100K protein binding and was not nonspecifically precipitated by antigen-antibody–protein A-Sepharose complexes.

The specificity with which the 100K protein bound to RNA was analyzed by adding various unlabeled competitors to binding reaction mixtures that contained ^{3}H-infected cell RNA. When the nucleic acid preference of the 100K protein was examined in this fashion, binding was found to be specific for single-stranded nucleic acids; a 50-fold excess of infected cell RNA or single-stranded DNA completely (100%) displaced ^{3}H-infected cell RNA binding, whereas tRNA, double-stranded DNA, and double-stranded RNA were ineffective competitors (0% displacement of ^{3}H-infected cell RNA). In this respect, the L4 100K protein resembles abundant RNA-binding proteins that are associated with RNA polymerase II transcripts (for example, see references 42, 43, and 59). Several members of this large

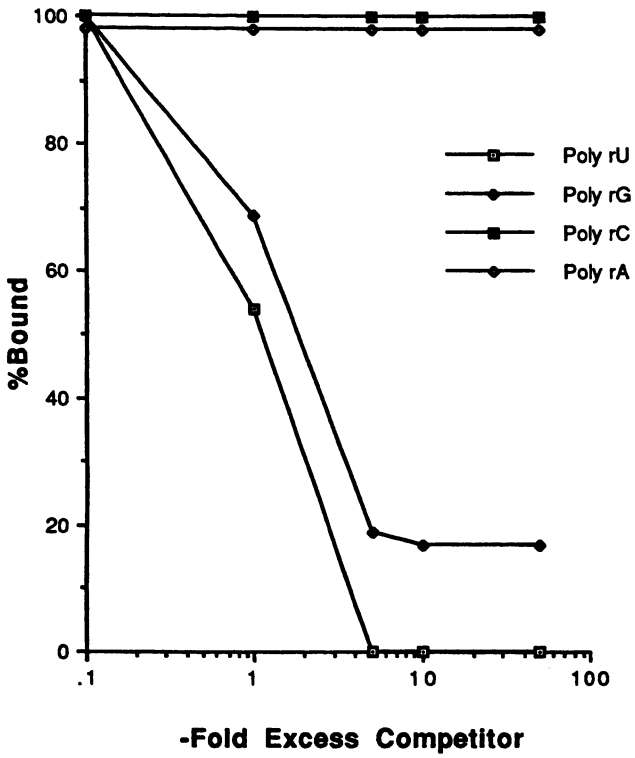

FIG. 3. Ribohomopolymer binding to the 100K protein. Competition by various ribohomopolymers for ^{3}H-infected cell RNA-100K protein binding was measured in immunoprecipitation assays as described in Materials and Methods. The added competitors are indicated in the inset, and the amounts of RNA bound are expressed as percentages of the amounts recovered in the absence of competitor.

class of cellular RNA-binding proteins exhibit strong preferences for specific ribohomopolymers. Poly(A)-binding proteins can, for example, be purified by virtue of their selective binding to poly(A) (8, 12), the heteronuclear ribonucleoprotein (hnRNP) C1 and C2 proteins bind preferentially to poly(U), and hnRNPs K and J bind very tightly to poly(C) (8, 12, 38, 53). Therefore, we employed the immunoprecipitation assay to determine whether the 100K protein bound preferentially to one or more ribohomopolymers. In these experiments, the efficiencies of binding to different RNAs were compared by competition assays in which increasing quantities of unlabeled RNA were added to binding reaction mixtures containing the 100K protein and ^{3}H-labeled, Ad5-infected cell RNA. Neither poly(C) nor poly(A) inhibited binding of the protein to infected cell RNA, even when present at a concentration 2 orders of magnitude higher than that of the labeled RNA probe (Fig. 3). By contrast, both poly(U) and poly(G) were effective inhibitors of the RNA-binding activity of the protein (Fig. 3). This difference in ribohomopolymer binding specificity was reproducibly observed with different preparations of the 100K protein. Some differences in mean length and ranges of lengths were observed when the ribohomopolymers were examined by electrophoresis under denaturing conditions. However, these differences cannot account for the results shown in Fig. 3; the poly(A) and poly(U) populations were, for example, similar, yet only the latter was bound efficiently by the 100K protein.

The results shown in Fig. 3 indicate that the adenovirus L4 100K protein possesses strong preferences for some RNA sequences (rich in U or G) over others. However, they do not address the most important question raised by our previous in vivo experiments (29), whether the 100K protein selectively recognizes viral late mRNA species. Therefore, we next compared the binding of the protein to cytoplasmic, poly(A)-containing and poly(A)-lacking RNAs prepared from both uninfected and Ad5-infected cells using the competition assay. Binding of the 100K protein to ^{3}H-infected cell RNA was decreased by addition of the unlabeled, homologous RNA and was completely inhibited by addition of a 10-fold (mass) excess of competitor (Fig. 4). However, no differences between poly(A)-containing and poly(A)-lacking RNA preparations or between uninfected and Ad5-infected cell RNA preparations as inhibitors of 100K binding to infected cell RNA could be discerned (Fig. 4).

Adenovirus infection induces inhibition of appearance in the cytoplasm of newly synthesized cellular mRNA and 28S rRNA (10, 15). Thus, most of the RNA labeled in infected cells between 18 and 22 h after infection used in these experiments should be viral in origin, particularly in the poly(A)-containing fraction. However, we wished to determine whether any specificity in the interaction of the 100K protein with RNA could be discerned when the protein was provided with a population of RNA molecules that was exclusively viral in origin. Therefore, we used SP6 RNA polymerase to transcribe a construct in which sequences encoding the 202-nucleotide tripartite leader upstream from an L2 mRNA for the 11-kDa precursor to virion protein μ (3) were placed downstream of the phage promoter. This approach also permitted comparison of capped and uncapped mRNAs as substrates for the 100K protein, a comparison that seemed particularly pertinent in view of the preference for G described previously. The results of these experiments were similar to those obtained when the specificity of 100K protein-RNA binding was examined with ^{3}H-infected cell RNA as probe; there was no difference in the displacement of ^{32}P-labeled L2 mRNA by capped or uncapped, unlabeled, synthetic L2 RNAs, and both uninfected and infected cell RNAs were effective inhibitors of the binding reaction (data not shown). Cellular RNA-binding proteins present in the 100K protein-containing cytoplasmic extracts of Ad5-infected cells do not register in the immunoprecipitation assay for the RNA-binding activity of the viral protein, as described previously. Nevertheless, it seemed possible that such cellular proteins interfered with our ability to detect preferences of the 100K protein for a particular class of infected cell RNA. Therefore, the 100K protein was purified by extraction of the pellet recovered upon centrifugation of cytoplasmic extracts at 100,000 × g for 1 h with 0.5 M NaCl. Analysis of the polypeptide compositions of such fractions by Coomassie blue staining of SDS-polyacrylamide gels established that the 100K protein constituted 10 to 20% of the total and was the most abundant polypeptide recovered, by a factor of 3 to 4 (data not shown). Nevertheless, no preference for viral poly(A)-containing RNA could be detected when such 100K protein preparations were used in competition experiments like those shown in Fig. 4.

In vitro RNA-binding activity of mutant and wild-type adenovirus 100K proteins correlates with in vivo translational efficiency. The ability of the 100K protein to bind to poly(U) (Fig. 3) allowed us to develop an assay with which to compare the effects of temperature on the RNA-binding activities of the wild-type and H5ts1 proteins; because temperature had been reported to affect antibody binding to

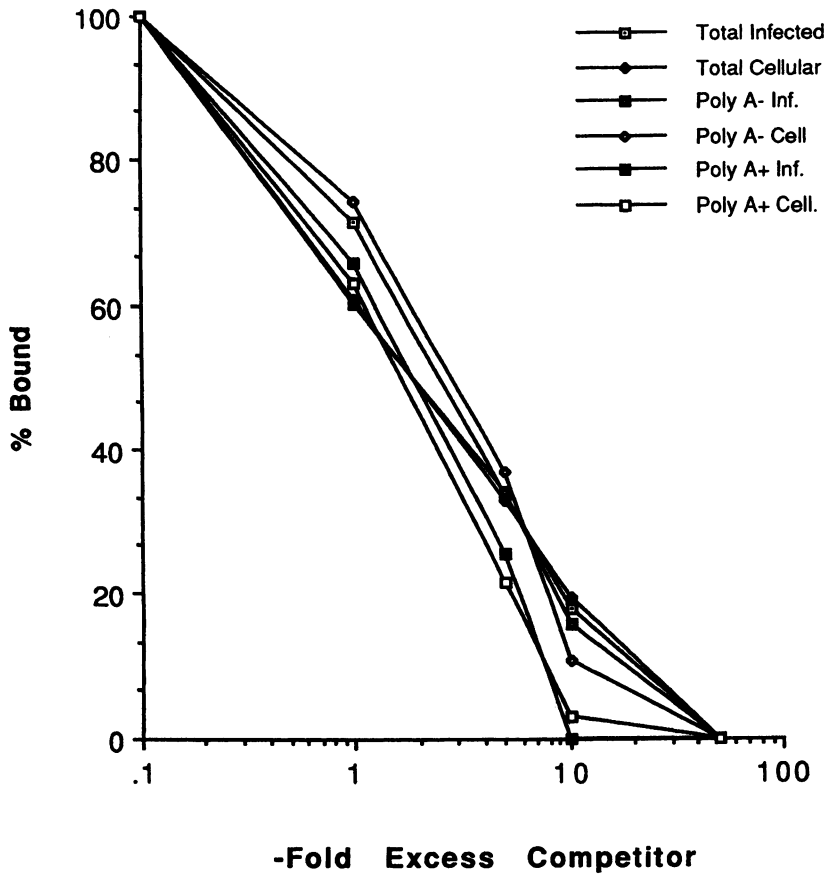

FIG. 4. Specificity of binding of the 100K protein to RNA. Competitors for ^{3}H-infected cell RNA-100K protein binding were added as indicated in the inset, and immunoprecipitations were performed as described in Materials and Methods. The quantities of RNA bound are expressed as percentages of those recovered when no competitor was added to binding reaction mixtures. Inf., infected cell; Cell., infected cell.

the mutant 100K protein (16), comparisons of the RNA-binding activities of wild-type and mutant proteins could not be made by the 2100K-1 immunoprecipitation assay. The binding activities of ^{35}S-labeled infected cell proteins to RNA were compared by using extracts prepared from cells infected by Ad5 or the H5ts1 mutant, which is translationally defective at 39°C (29). Total cell proteins (Fig. 7, lanes 2 and 3) prepared from wild-type- and mutant-infected cells at the permissive temperature (33°C) were incubated with poly(U) RNA immobilized on Sepharose. Two major labeled proteins were eluted from the poly(U)-Sepharose (Fig. 5, lanes 1 and 2). The identities of these two proteins as the 100K protein and late polypeptide II were confirmed by eluting the poly(U)-Sepharose with 0.3 M NaCl in RBB and immunoprecipitating the eluted proteins with the 100K protein-specific antibody 2100K-1 (Fig. 5, lanes 5 and 6). To determine whether the abundant polypeptide II was present in these RNA eluates by virtue of its association with the 100K protein or as a result of direct RNA binding, protein eluates were rebound and eluted from the poly(U)-Sepharose (Fig. 5, lanes 3 and 4). Polypeptide II was present after two rounds of poly(U) selection, indicating that it is unlikely to be nonspecifically trapped by the poly(U)-Sepharose; it is probably bound to the RNA as a result of its interaction with the 100K protein.

The purified 100K protein obtained by chromatography on poly(U)-Sepharose consistently migrated as a doublet, whether labeled 100K protein present in the eluate was analyzed directly by SDS-PAGE or following immunoprecipitation with 2100K-1 antibody (Fig. 5, lanes 1, 2, 5, and 6). Because the 100K protein is highly phosphorylated (see the Introduction), the two species of the 100K protein that can be separated in these experiments presumably are differentially phosphorylated forms. The two species apparent in purified 100K protein preparations (Fig. 5) cannot readily be distinguished in total cytoplasmic extracts (for example, see Fig. 7, lanes 2 and 3), presumably because of the presence of a large number of other labeled proteins.

The in vitro temperature sensitivities of wild-type and mutant 100K proteins were analyzed by comparing binding to RNA at 33 and 39°C, the permissive and nonpermissive temperatures, respectively, for H5ts1. In these experiments, labeled cytoplasmic proteins were prepared following infection at the permissive temperature, 33°C. Therefore, similar quantities of 100K protein were labeled in wild-type- and H5ts1 mutant-infected cells (Fig. 7, lanes 2 and 3). The 100K protein present in extracts prepared from Ad5-infected cells maintained at the permissive temperature bound well to poly(U)-Sepharose at both 33 and 39°C (Fig. 6, lanes 1, 4, 5, and 6). By contrast, binding of the 100K protein prepared from H5ts1-infected cells was significantly less at 39°C than at 33°C (Fig. 6, compare lanes 7 and 8 with lanes 2 and 3).

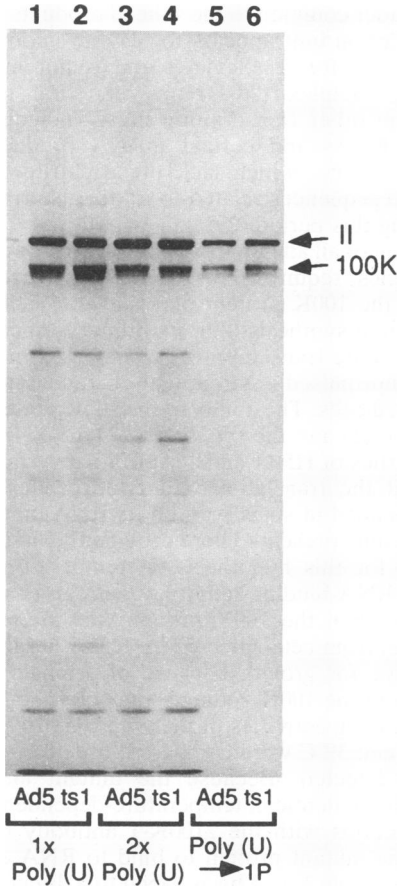

FIG. 5. Binding of the 100K protein to poly(U)-Sepharose. ^{35}S-labeled cytoplasmic proteins were bound and eluted from poly(U)-Sepharose as described in Materials and Methods (lanes: 1, Ad5-infected cells; 2, H5ts1-infected cells). Proteins eluted with 0.3 M NaCl were either rebound and eluted from poly(U)-Sepharose (lanes: 3, Ad5; 4, H5ts1) or immunoprecipitated (1P) with the 100K protein antibody 2100K-1 (lanes: 5, Ad5; 6, H5ts1).

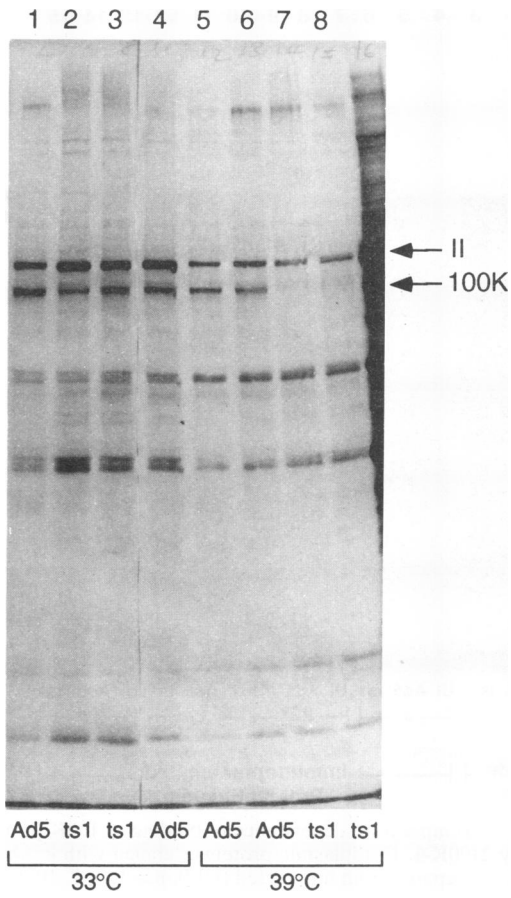

FIG. 6. Temperature dependence of binding of Ad5 and H5ts1 100K proteins to RNA. Cytoplasmic proteins labeled with [^{35}S]methionine were prepared from Ad5-infected or H5ts1-infected cells harvested late in infection, at 33°C. Following incubation with poly(U)-Sepharose at 33°C (lanes 1 and 4, Ad5; lanes 2 and 3, H5ts1) or 39°C (lanes 5 and 6, Ad5; lanes 7 and 8, H5ts1), bound proteins were eluted, separated by SDS-PAGE, and visualized by fluorography.

Quantitation of the results shown in Fig. 6 and of those from similar experiments with independent preparations of the 100K proteins (Table 1) established that the RNA-binding activity of the wild-type protein was decreased by no more than 1.6-fold at 39°C. By contrast, when incubated at 39°C, H5ts1 100K protein prepared at low temperature exhibited a 3.7- to 4.4-fold decrease in RNA-binding activity (Table 1).

The decreased ability of the H5ts1 100K protein to bind to RNA at 39°C could be the direct result of its temperature-dependent loss of RNA-binding activity or a trivial result of the increased instability of the mutant protein at high temperature. To investigate the latter possibility, we compared the temperature sensitivities of the interactions of the wild-type and mutant 100K proteins with 2100K-1 antibody. No difference between the binding activities of the wild-type and H5ts1 mutant proteins to the antibody was observed; similar quantities of the wild-type and mutant 100K polypeptides were recovered following immunoprecipitation of infected cell extracts at 4, 22, or 33°C (Fig. 7, lanes 4 to 12). In both cases, moreover, incubation at 39°C resulted in a decrease in the quantity of 100K protein immunoprecipitated (Fig. 7, lanes 13 to 15). Under the same conditions, no cellular

TABLE 1. Quantitation of Ad5 and H5ts1 100K proteins[a]

Measurement no.	Concn of 100K protein				Concn ratios for 100K protein[b]	
	Ad5		H5ts1		Ad5	H5ts1
	33°C	39°C	33°C	39°C		
1	0.56	0.43	0.57	0.15	1.30	3.80
2	0.53	0.40	0.53	0.12	1.33	4.42
3	0.67	0.40	0.67	0.18	1.67	3.72

[a] Gel scanning and image analysis were performed with a Microtex Scanner 60025 and the Macintosh programs Adobe photoshop and NIH image analysis. The area of each 100K protein peak, in gels like that shown in Fig. 6, is given in arbitrary units. Measurements 1 and 2 were made from the autoradiogram shown in Fig. 6. Measurement 3 was from a separate experiment with independently prepared proteins. Qualitatively similar results were observed in a third independent experiment but could not be accurately quantitated because of high, nonspecific background in some lanes.

[b] Ratios are of concentrations at 33°C to concentrations at 39°C.

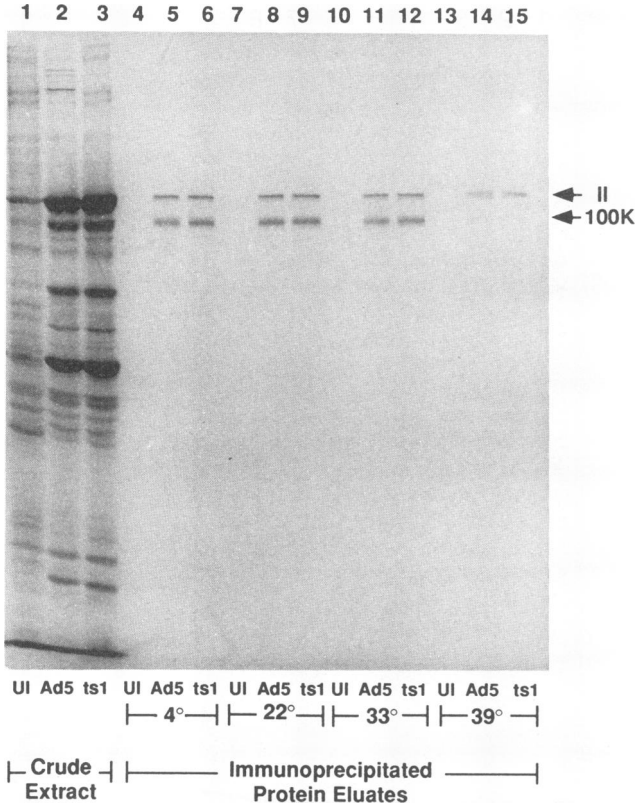

FIG. 7. Temperature dependence of binding of 100K proteins to antibody 2100K-1. Cytoplasmic proteins, labeled with [^{35}S]methionine, were prepared from uninfected (UI [lanes 1, 4, 7, 10, and 13]), Ad5-infected (lanes 2, 5, 8, 11, and 14), or H5ts1-infected (lanes 3, 6, 9, 12, and 15) cells late in infection at 33°C. Following incubation at 4°C (lanes 4, 5, and 6), 22°C (lanes 7, 8, and 9), 33°C (lanes 10, 11, and 12), or 39°C (lanes 13, 14, and 15), the antibody 2100K-1 was added, and immunoprecipitation was performed as described in Materials and Methods.

proteins were immunoprecipitated (Fig. 7, lanes 4, 7, 10, and 13).

DISCUSSION

Adenovirus infection alters the translational machinery of cells permissive for its replication, impairing the activity of at least two initiation factors. Although the virus-encoded RNA, VA1 RNA, partially circumvents the phosphorylation of the α subunit of EIF-2 and concomitant inactivation of this factor resulting from activation of EIF-2 kinase (see the introduction), the degree of phosphorylation that can be attained even in the presence of VA1 RNA is characteristic of complete inhibition of translation in other systems (39). In addition, EIF-4F (cap-binding complex) is inactivated following adenovirus infection (31). Thus, by the late phase of infection, essential components of the cellular translational initiation machinery are functionally impaired. Despite such a translationally compromised environment, viral late mRNA species continue to be translated for many hours. Because cellular mRNAs are present in infected cells throughout this period and are translatable in vitro (6, 56), it is clear that a mechanism must exist to ensure translation of a specific subset of the mRNAs present in the cytoplasm of cells that have entered the late phase of infection. The tripartite leader common to the spliced products of the major late transcription unit appears to play an important role in such selection, for it alleviates the requirement for the cap-binding complex (20). However, viral mRNAs expressed from other transcription units, including the IVa_2 and pIX mRNAs and certain mRNA products of early transcription units, which lack the tripartite leader or 5′ untranslated sequences related to it, are also translationally active during this period (29).

Translation of all adenovirus late mRNAs, but not of early mRNA species, requires the L4 100K nonstructural protein (29). Since the 100K protein plays no role in inhibition of cellular protein synthesis (29), its primary function appears to be to facilitate translation of late mRNAs in the translationally compromised environment characteristic of adenovirus-infected cells. The ability of the 100K protein to bind to RNA in vivo (1) and the sequences (24, 29, 34) and phenotypic properties of H5ts1 and a set of its revertants led us to suggest that the translational function of this adenovirus protein depended in some way on its RNA-binding activity (29). The results presented here provide the first experimental support for this hypothesis. A temperature-dependent decrease in RNA-binding activity was observed in vitro (Fig. 6; Table 1) when the 100K protein was prepared (at low temperature) from cells infected by H5ts1, a virus temperature sensitive for growth because of a single amino acid substitution in the 100K coding sequence (29). By contrast, when the binding activities of the wild-type 100K protein to RNA at 33 and 39°C were compared, little effect of temperature was detected. Because the mutant and wild-type proteins exhibit identical, temperature-dependent changes in their interactions with the 2100K-1 antibody (Fig. 7), the failure of the mutant protein to bind to RNA efficiently at 39°C (Fig. 6; Table 1) can be ascribed to a direct effect of the mutation rather than an indirect effect resulting from changes in protein stability. Thus, the in vitro temperature sensitivity of H5ts1 100K protein RNA-binding activity correlates with in vivo translational efficiency; both the in vitro RNA-binding activity of the mutant protein and in vivo translational efficiency are lower at the nonpermissive temperature (39°C) than at the permissive temperature (33°C). The wild-type and the mutant 100K proteins undergo a common conformational change in vitro when the temperature is raised from 33 to 39°C that results in loss of recognition of the 2100K-1 epitope (Fig. 7), but neither the in vivo translational function nor the in vitro RNA-binding activity of the wild-type protein is significantly impaired at 39°C (29) (Fig. 6; Table 1). Therefore, it appears that some aspect of the conformation of the wild-type protein is intrinsically sensitive to temperature and that an independent, temperature-sensitive change resulting from replacement of serine 466 by proline in the H5ts1 100K protein (29) leads to loss of translational function in vivo (29) and to reduced RNA-binding activity in vitro. In vivo, little recovery from the inhibitory effects of the H5ts1 mutation is observed in the first few hours following a shift from a nonpermissive to a permissive temperature (28a), suggesting that this conformational change is irreversible.

In view of the properties of translation during the late phase of adenovirus infection discussed previously, the most obvious function for the RNA-binding activity of the 100K protein would be in direct selection of late mRNAs for efficient translation. Viral or cellular proteins that control the translation of specific mRNA species have been previously identified and characterized and in some cases have been shown to be sequence-specific RNA-binding proteins (9, 44).

The Rev protein of the human immunodeficiency virus (HIV) and the cellular iron-responsive element (IRE) binding protein, for example, interact specifically with *cis*-acting RNA elements, the Rev-response element (RRE) and IRE, respectively, that contain stem-loop structures (4, 19, 27, 36, 46). Interaction of the IRE binding protein with an IRE in the 5′ untranslated region of ferritin mRNA suppresses translation (46), whereas binding of Rev to RREs present in unspliced and partially spliced HIV mRNAs facilitates their assembly into polyribosomes and translation (4, 19). Although the adenovirus 100K protein, like Rev, increases polysome loading and translation of a specific set of mRNA species (29), it exhibited no preference for binding to viral mRNAs in our in vitro assays (e.g., Fig. 4). It might be argued that the L4 100K protein requires features absent from in vitro binding assays. Such features might include specific secondary or tertiary structures, a structure stabilized by other RNA-binding proteins, or interaction of the 100K protein with cellular factors. It seems unlikely that an essential protein that interacted with either viral mRNA or the L4 100K protein was omitted from the in vitro binding reactions; the extracts used in these experiments contained many RNA-binding proteins detected by mobility shift assays, addition of uninfected cell cytoplasmic proteins to binding reactions did not alter the RNA-binding properties of the 100K protein, and no differences were observed when the RNA-binding properties of the 100K protein purified to different degrees were compared (data not shown). Furthermore, the 100K protein has been shown to bind to both viral and cellular RNA sequences in vivo (1), as it does in our in vitro assays. Therefore, it seems unlikely that the specificity with which the 100K protein increases translational efficiency in adenovirus-infected cells (21) is the result of specific binding of this protein to viral late mRNAs.

We have previously reported (29) that of the H5ts1 100K and H5ts2 hexon mutations, only the former induces a significant translational defect under nonpermissive conditions, despite their similar phenotypes with respect to hexon morphogenesis (16). Therefore, the translational function of the 100K protein appears to be independent of its role in hexon trimerization, which is presumed to be mediated by interaction with nascent hexon polypeptide chains (e.g., see references 16, 25, and 40). Nevertheless, the presence of hexon polypeptide II in all preparations of the 100K protein used in our RNA-binding assays raises the question of whether the former protein modulates the RNA-binding activity of the latter. Expression of the 100K protein in a heterologous system; to ensure that it is free from even trace quantities of polypeptide II (and vice versa), will be necessary to provide a definitive answer to this question. In the meantime, several lines of evidence indicate that the RNA-binding properties reported here are those of the L4 100K protein itself. Thus, for example, the H5ts1 mutation, which has been identified as a single amino acid substitution of amino acid 466 of the 100K protein (29), renders RNA binding temperature sensitive (Fig. 6). Furthermore, Adam and Dreyfuss (1) have demonstrated that RNA labeled in adenovirus-infected cells becomes covalently attached to the 100K protein upon exposure of intact cells to UV light and thus that the 100K protein is in direct contact with RNA in vivo. In these experiments, no evidence for RNA binding by hexon polypeptide II was reported. Finally, as discussed previously, 100K protein preparations exhibiting different ratios of hexon polypeptide II to the 100K protein, ranging from excess hexon to a large excess of the 100K protein, exhibited no detectable differences in RNA-binding specificities in our in vitro assays.

Although the Ad5 100K protein failed to distinguish viral from cellular mRNAs or poly(A)-containing from poly(A)-lacking RNAs in in vitro binding assays, it exhibited a striking preference for poly(U) or poly(G) over poly(A) or poly(C) (Fig. 3). Several cellular RNP-binding proteins have previously been shown to exhibit strong preferences for specific ribohomopolymers, although in most cases such specificity is most evident when RNA-binding assay mixtures contain high salt concentrations (e.g., see reference 53). The hnRNP C proteins, for example, exhibit a strong preference for poly(U) (53) and bind to uridine-rich polypyrimidine sequences present at the 3′ ends of introns and downstream of polyadenylation signals in pre-mRNA (54, 62, 63). Such binding specificity is in accord with the participation of the C protein in pre-mRNA processing in vitro (17, 51). Therefore, it seems reasonable to suppose that the ribohomopolymer preference exhibited by the adenoviral 100K protein in vitro is related to its in vivo function. However, a function for the 100K protein in RNA processing analogous to that of the RNP C proteins seems unlikely; cells infected by H5ts1 at a nonpermissive temperature display no discernible defects in the production of viral mRNA species (29). Moreover, unlike the cellular RNP C proteins (53), the Ad5 100K protein does not bind poly(U) efficiently at high salt concentrations (Fig. 5).

It is possible that the RNA-binding activity of the L4 100K protein is related to the mechanism (presently unknown) whereby the protein facilitates translation. If this were the case, the 100K protein could be considered a general translational activator, the specificity observed in vivo being conferred by other components. Such components would include the tripartite leader, which renders translation of mRNAs to which it is linked independent of EIF-4F (20), and VA1 RNA, which has been proposed to confer local protection against inactivation of EIF-2α in the vicinity of viral late mRNA species and thus ensure the continued translation of this class (39). Although we believe that the L4 100K protein cannot select viral late mRNAs for translation by sequence-specific binding to them, it remains possible that the protein identifies viral late mRNAs by virtue of some other distinctive property common to this class. The most likely candidate for such a property is the selective export of these mRNAs from the nucleus to the cytoplasm (e.g., see references 7, 10, 15, 41, and 61). From about 12 h after infection of permissive cells, viral late mRNAs are the only newly synthesized mRNA species entering the cytoplasm and could thus be selectively recognized by the L4 100K protein, despite its inability to bind to RNA in a sequence-specific fashion. Evidence for such gating (13) is accumulating from studies in other systems. For example, it has been proposed that the efficient translation of heat shock proteins in cells exposed to inducers of the stress response is related to preferential production of heat shock mRNAs (57). The properties of the HIV Rev protein provide more direct evidence for such a mechanism; this protein not only facilitates translation of specific mRNA species, as discussed previously, but also the export of these mRNAs from the nucleus to the cytoplasm (21, 22, 36). Thus, the HIV Rev protein ensures both efficient production and efficient utilization in the cytoplasm of a subset of HIV mRNAs. If a similar mechanism operated in adenovirus-infected cells, the export and translation functions would appear to be divided among different viral proteins; no defect in export of viral mRNA from the nucleus is observed in H5ts1-infected cells

at a nonpermissive temperature (29), and the selective export of viral mRNAs during the late phase of infection requires early E1B and E4 proteins (7, 14, 18, 41, 61).

Although consistent with the RNA-binding properties of the L4 100K protein reported here, the proposition that this protein directs efficient translation of mRNA species that enter the cytoplasm during the late phase of infection is not presently supported by direct experimental evidence. Nor does this model address the question of how the L4 protein might facilitate initiation of translation, once specific mRNAs have been selected. Thus, additional experiments are required to establish the biological function of the apparently nonspecific RNA-binding activity of this adenovirus protein.

ACKNOWLEDGMENT

This work was supported by a grant from the USPHS (AI17265) to S.J.F.

REFERENCES

1. **Adam, S., and G. Dreyfuss.** 1987. Adenovirus proteins associated with mRNA and hnRNA in infected HeLa cells. J. Virol. **61**:3276–3283.
2. **Alonso-Caplen, F. V., M. G. Katze, and R. M. Krug.** 1988. Efficient transcription not translation is dependent on adenovirus tripartite leader sequences at late times of infection. J. Virol. **62**:1606–1616.
3. **Anderson, C. W., M. E. Young, and S. J. Flint.** 1989. Characterization of the adenovirus 2 virion protein, mu. Virology **172**:506–512.
4. **Arrigo, S. J., and I. S. Y. Chen.** 1991. Rev is necessary for translation but not cytoplasmic accumulation of HIV-1 *vif*, *vpr*, and *env/vpu 2* RNAs. Genes Dev. **5**:808–819.
5. **Axelrod, N.** 1978. Phosphoproteins of adenovirus 2. Virology **87**:366–383.
6. **Babich, A., C. T. Feldman, J. R. Nevins, J. E. Darnell, and C. Weinberger.** 1983. Effect of adenovirus on metabolism of specific host mRNAs; transport control and specific translational discrimination. Mol. Cell. Biol. **3**:1212–1221.
7. **Babiss, L. E., and H. S. Ginsberg.** 1984. Adenovirus type 5 early region 1b gene product is required for efficient shutoff of host protein synthesis. J. Virol. **50**:202–212.
8. **Baer, B. W., and R. D. Kornberg.** 1983. The protein responsible for the repeating structure of cytoplasmic poly (A) ribonucleoprotein. J. Cell. Biol. **96**:717–721.
9. **Bandziulis, R. J., M. S. Swanson, and G. Dreyfuss.** 1989. RNA-binding proteins as developmental regulators. Genes Dev. **3**:431–437.
10. **Beltz, G. A., and S. J. Flint.** 1979. Inhibition of HeLa cell protein synthesis during adenovirus infection: restriction of cellular messenger RNA sequences to the nucleus. J. Mol. Biol. **131**:353–373.
11. **Berkner, K. L., and P. A. Sharp.** 1985. Effect of tripartite leader on synthesis of a non-viral protein in an adenovirus 5 recombinant. Nucleic Acids Res. **13**:841–857.
12. **Blanchard, J. M., C. Brissac, and P. Jeanteur.** 1974. Characterization of a protein species isolated from HeLa cell cytoplasm by affinity chromatography on polyadenylate-Sepharose. Proc. Natl. Acad. Sci. USA **71**:1882–1886.
13. **Blobel, G.** 1985. Gene gating: a hypothesis. Proc. Natl. Acad. Sci. USA **82**:8527–8529.
14. **Bridge, E., and G. Ketner.** 1990. Interaction of adenoviral E4 and E1b products in late gene expression. Virology **174**:345–353.
15. **Castiglia, C. L., and S. J. Flint.** 1983. Effects of adenovirus infection on rRNA synthesis and maturation in HeLa cells. Mol. Cell. Biol. **3**:662–671.
16. **Cepko, C. L., and P. A. Sharp.** 1983. Analysis of Ad5 hexon and 100k ts mutants using conformation-specific monoclonal antibodies. Virology **129**:137–154.
17. **Choi, Y. D., P. J. Grabowski, P. A. Sharp, and G. Dreyfuss.** 1986. Heterogeneous nuclear ribonucleoproteins: role in RNA splicing. Science **231**:1534–1539.
18. **Cutt, J. R., T. Shenk, and P. Hearing.** 1987. Analysis of adenovirus early region 4-encoded polypeptides synthesized in productively infected cells. J. Virol. **61**:543–552.
19. **D'Agostino, D. M., B. K. Felber, J. E. Harrison, and G. N. Pavlakis.** 1992. The Rev protein of human immunodeficiency virus type 1 promotes polysomal association and translation of *gag/pol* and *vpu/env* mRNAs. Mol. Cell. Biol. **12**:1375–1386.
20. **Dolph, P. J., V. Racaniello, A. Villamarin, F. Pallodino, and R. J. Schneider.** 1988. The adenovirus tripartite leader may eliminate the requirement of cap-binding protein complex during translation initiation. J. Virol. **62**:2059–2066.
21. **Emerman, M., R. Vazeux, and K. Peden.** 1990. The rev gene product of the human immunodeficiency virus affects envelope-specific RNA localization. Cell **57**:1155–1165.
22. **Felber, B. K., M. Hadzopoulou-Cladaras, C. Cladaras, T. Copeland, and G. N. Pavlakis.** 1989. Rev protein of human immunodeficiency virus type 1 affects the stability and transport of the viral mRNA. Proc. Natl. Acad. Sci. USA **86**:1495–1499.
23. **Flint, S. J.** 1984. Adenovirus cytopathology. Compr. Virol. **19**:297–358.
24. **Gambke, C., and W. Deppert.** 1981. Late nonstructural 100,000- and 33,000-dalton proteins of adenovirus type 2. I. Subcellular localization during the course of infection. J. Virol. **40**:585–593.
25. **Gambke, C., and W. Deppert.** 1983. Specific complex of the late nonstructural 100k protein with newly synthesized hexon in adenovirus type 2-infected cells. Virology **124**:1–12.
26. **Ginsberg, H. S.** 1984. The adenoviruses. Plenum Press, New York.
27. **Goossen, B., S. W. Caughman, J. B. Harford, R. D. Klausner, and M. W. Hentze.** 1990. Translational repression by a complex between the iron responsive element of ferritin mRNA and its specific cytoplasmic binding protein is position-dependent *in vivo*. EMBO J. **9**:4127–4133.
28. **Graham, F. L., J. Smiley, W. C. Russell, and R. Nairn.** 1977. Characteristics of a human cell-line transformed by DNA from adenovirus type 5. J. Gen. Virol. **36**:59–72.

28a. **Hayes, B. W., and S. J. Flint.** Unpublished observations.

29. **Hayes, B. W., G. C. Telling, M. M. Myat, J. F. Williams, and S. J. Flint.** 1990. The adenovirus L4 100-kilodalton protein is necessary for efficient translation of viral late mRNA species. J. Virol. **64**:2732–2742.
30. **Huang, J., and R. J. Schneider.** 1990. Adenovirus inhibition of cellular protein synthesis is prevented by the drug 2-aminopurine. Proc. Natl. Acad. Sci. USA **87**:7115–7119.
31. **Huang, J., and R. J. Schneider.** 1991. Adenovirus inhibition of cellular protein synthesis involves inactivation of cap-binding protein. Cell **65**:271–280.
32. **Katze, M. G., D. DeCorato, B. Safer, J. Galabru, and A. G. Hovanessian.** 1987. Adenovirus VA1 RNA complexes with the 68,000 Mr protein kinase to regulate its autophosphorylation and activity. EMBO J. **6**:689–697.
33. **Kaufman, R. J.** 1985. Identification of the components necessary for adenovirus translational control and their utilization in cDNA expression vectors. Proc. Natl. Acad. Sci. USA **82**:689–693.
34. **Lindberg, U., and B. Sundquist.** 1974. Isolation of messenger ribonucleoproteins from mammalian cells. J. Mol. Biol. **86**:451–468.
35. **Logan, J., and T. Shenk.** 1984. Adenovirus tripartite leader sequence enhances translation of mRNAs late after infection. Proc. Natl. Acad. Sci. USA **81**:3655–3659.
36. **Malimo, M. H., J. Hauber, S. Le, J. V. Maizel, and B. R. Cullen.** 1989. The HIV-1 rev transactivator acts through a structured sequence to activate nuclear export of unspliced viral mRNA. Nature (London) **338**:254–257.
37. **Mathews, M. B., and T. Shenk.** 1991. Adenovirus virus-associated RNA and translation control. J. Virol. **65**:5657–5662.
38. **Matunis, M. J., W. M. Michael, and G. Dreyfuss.** 1992. Characterization and primary structure of the poly(C)-binding heterogeneous nuclear ribonucleoprotein complex K protein. Mol. Cell. Biol. **12**:164–171.

39. **O'Malley, R. P., R. F. Duncan, J. W. B. Hershey, and M. B. Mathews.** 1989. Modification of protein synthesis initiation factors and the shut-off of host protein synthesis in adenovirus-infected cells. Virology **168:**112–118.
40. **Oosterom-Dragon, E. A., and H. S. Ginsberg.** 1981. Characterization of two temperature-sensitive mutants of type 5 adenovirus with mutations in the 100,000-dalton protein gene. J. Virol. **40:**491–500.
41. **Pilder, S., J. Logan, and T. Shenk.** 1986. The adenovirus E1B-55K transforming polypeptide modulates transport or cytoplasmic stabilization of viral and host cell mRNAs. Mol. Cell. Biol. **6:**470–476.
42. **Piñol-Roma, S., M. S. Swanson, M. J. Matunis, and G. Dreyfuss.** 1990. Purification and characterization of proteins of heterogeneous nuclear ribonucleoprotein complexes by affinity chromatography. Methods Enzymol. **181:**326–331.
43. **Pullman, J. M., and T. E. Martin.** 1983. Reconstitution of nucleoprotein complexes with mammalian heterogeneous nuclear ribonucleoprotein (hnRNP) core proteins. J. Cell Biol. **97:**99–111.
44. **Query, C. C., R. C. Bentley, and J. D. Keene.** 1989. A common RNA recognition motif identified within a defined U1 RNA binding domain of the 70K U1 snRNP protein. Cell **57:**89–101.
45. **Reichel, P. A., W. C. Merrick, J. Siekierka, and M. B. Mathews.** 1985. Regulation of a protein synthesis initiation factor by adenovirus virus-associated RNA. Nature (London) **313:**196–200.
46. **Rouault, T. A., M. W. Hentze, S. W. Caughman, J. B. Harford, and R. D. Klausner.** 1988. Binding of a cytosolic protein to the iron-responsive element of human ferritin messenger RNA. Science **241:**1207–1210.
47. **Russell, W. C., and G. E. Blair.** 1977. Polypeptide phosphorylation in adenovirus-infected cells. J. Gen. Virol. **34:**19–35.
48. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed., vol. 3, p. 7.12–7.16. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
49. **Schneider, R. J., B. D. Safer, S. M. Munemitsu, C. E. Samuel, and T. Shenk.** 1985. Adenovirus VA1 RNA prevents phosphorylation of the eukaryotic initiation factor 2 alpha subunit subsequent to infection. Proc. Natl. Acad. Sci. USA **82:**4321–4325.
50. **Schneider, R. J., and T. Shenk.** 1987. Impact of virus infection on host cell protein synthesis. Annu. Rev. Biochem. **56:**317–332.
51. **Sierakowska, H., W. Szer, P. J. Furdon, and R. Kole.** 1986. Antibodies to hnRNP core proteins inhibit *in vitro* splicing of human β-globin pre-mRNA. Nucleic Acids Res. **14:**5241–5254.
52. **Sundquist, B., T. Persson, and U. Lindberg.** 1977. Characterization of mRNA-protein complexes from mammalian cells. Nucleic Acids Res. **4:**899–915.
53. **Swanson, M. S., and G. Dreyfuss.** 1988. Classification and purification of proteins of heterogeneous nuclear ribonucleoprotein particles by RNA-binding specificities. Mol. Cell. Biol. **8:**2237–2241.
54. **Swanson, M. S., and G. Dreyfuss.** 1988. RNA binding specificity of hnRNP proteins: a subset bind to the 3′ end of introns. EMBO J. **7:**3519–3529.
55. **Tasserron-de Jong, J. G., J. Brouwer, K. Rietveld, C. E. M. Zoetemelk, and L. Bosch.** 1979. Messenger ribonucleoprotein complexes in human KB cells infected with adenovirus type 5 contain tightly bound viral-coded "100K" proteins. Eur. J. Biochem. **100:**271–283.
56. **Thimmappaya, B., C. Weinberger, R. J. Schneider, and T. Shenk.** 1982. Adenovirus VA1 RNA is required for efficient translation of viral mRNAs at late times after infection. Cell **31:**543–551.
57. **Thomas, G. P., and M. B. Mathews.** 1984. Alterations of transcription and translation in HeLa cells exposed to amino acid analogs. Mol. Cell. Biol. **4:**1063–1072.
58. **Van der Marel, P., J. G. Tasseron-de Jong, and L. Bosch.** 1975. The proteins associated with mRNA from uninfected and adenovirus type 5 infected KB cells. FEBS Lett. **51:**330–334.
59. **Wilk, B.-E., G. Angeli, and K. P. Schafer.** 1983. *In vitro* reconstitution of 35S ribonucleoprotein complexes. Biochemistry **22:**4592–4600.
60. **Williams, J. F., M. Gharpure, S. Ustacelebi, and S. McDonald.** 1971. Isolation of temperature-sensitive mutants of adenovirus type 5. J. Gen. Virol. **11:**95–101.
61. **Williams, J. F., B. D. Karger, Y.-S. Ho, C. L. Castiglia, T. Mann, and S. J. Flint.** 1986. The adenoviral E1B 495R protein plays a role in regulation of the exit of mRNA from the infected cell nucleus and in determining the stability of viral, late mRNA in the cytoplasm. Cancer Cells (Cold Spring Harbor) **4:**275–284.
62. **Wilusz, J., D. I. Feig, and T. Shenk.** 1988. The C proteins of heterogeneous nuclear ribonucleoprotein complexes interact with RNA sequences downstream of polyadenylation cleavage sites. Mol. Cell. Biol. **8:**4477–4483.
63. **Wilusz, J., and T. Shenk.** 1990. A uridylate tract mediates efficient heterogeneous ribonucleoprotein C protein-RNA crosslinking and functionally substitutes for the downstream element of the polyadenylation signal. Mol. Cell. Biol. **10:**6397–6407.