Specific Interaction *in Vitro* and *in Vivo* of Glyceraldehyde-3-phosphate Dehydrogenase and LA Protein with Cis-acting RNAs of Human Parainfluenza Virus Type 3*

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Human parainfluenza virus type 3 (HPIV3), belonging to the paramyxovirus family, is one of the major causes of pneumonia and bronchiolitis in infants (1). HPIV3 contains a negative strand RNA genome that is encapsidated by a nucleocapsid protein NP (68 kDa) and tightly associated with two RNA polymerase subunits, a large protein L (251 kDa) and a phosphoprotein P (90 kDa), to form the viral ribonucleoprotein (RNP) core (2, 3). The encapsidated genome RNA serves as a template for transcription to synthesize a leader RNA and six mRNAs as well as in replication to synthesize full-length genome RNA, both mediated by the viral RNA-dependent RNA polymerase. Recent studies demonstrate that participation of specific cellular proteins is critical for the regulation of gene expression of HPIV3 (4, 5). Protein kinase C-ζ has been implicated in the phosphorylation of the virion-associated RNA polymerase subunit, the phosphoprotein P (5). Introduction of protein kinase C-ζ-specific peptide inhibitor in cultured cells abrogated HPIV3 replication providing strong evidence that protein kinase C-ζ is involved in the HPIV3 life cycle (5). Another cellular protein, actin, was found to be required in transcription of purified viral RNP *in vitro* and was found to be involved in maintaining a moderately coiled structure of the RNP that appeared to facilitate transcription of the genome RNA by the RNA polymerase (4). The productive infection of HPIV3, thus, appears to require a close encounter between the viral genome and several cellular proteins. A detailed search of such putative cellular proteins and their characterization would lead to better understanding of their roles in the regulation of the intricate steps in viral gene expression.

Sequence analysis of HPIV3 genome RNA reveals the presence of a sequence element at the 3′-end that serves as the binding site of the RNA polymerase to initiate synthesis of a 55-nucleotide plus-sense leader RNA followed by six monocistronic, capped, and polyadenylated mRNAs *in vitro* and *in vivo* (2, 3). The leader RNA is involved in the initiation of assembly of viral nucleocapsid containing the plus-sense genome RNA that in turn serves as a template for the synthesis of minus strand genome RNA for packaging into progeny virions (6). Thus, the 3′-noncoding region of the genome RNA and the plus-sense leader RNA are the key cis-acting RNA sequence regions that presumably play important roles in the regulation of virus transcription and replication, respectively. A number of observations suggest that cellular proteins specifically interact with the viral cis-acting regulatory RNAs in several viral systems indicating their possible involvement in the regulation of viral gene expression (7–12).

In this study, we searched for putative cellular proteins that might be involved in HPIV3 gene expression through interaction with the viral cis-acting regulatory RNAs. In a gel mobility shift assay, we used two regulatory RNAs described above, the 3′-genome sequence-containing RNA (3′-GS-RNA) and the leader sequence-containing RNA (LS-RNA) that are involved in binding of RNA polymerase and the viral nucleocapsid protein for transcription and replication, respectively. We have shown that the cellular glycolytic enzyme, GAPDH, and the nuclear antigen, LA protein, form specific complexes with these cis-acting RNAs *in vitro* and *in vivo*. In addition, GAPDH is found to be co-localized with the viral RNP in HPIV3-infected cells. These results strongly suggest that both GAPDH and LA proteins are involved in the regulation of gene expression of HPIV3.

EXPERIMENTAL PROCEDURES

Plasmid Constructs and *in Vitro* Transcription—Two plasmids containing the oligodeoxynucleotides corresponding to a 73-nucleotide 3′-genome sequence (3′-GS) and plus-sense LS-RNA, respectively, under
the control of T7 promoter were constructed. To construct the plasmid-
containing 1 (5 ’-AAG GAC GAT TAG TCA ATG TCT ATC C-3’) containing a HindIII site, T7 promoter complementary 
sequence, and 17 nucleotides from the untranslated region of the NP gene and primer 2 (5 ’-GTT ACC GAC GCT ATA TAC CAA AGA GAA GAA ACT TG-3’) containing KpnI-HgaI sites and a 22-nucleotide complementary sequence from the 3’-end of the leader region were synthesized (Operon Technologies, Inc.). These two primers were annealed in vivo to form the chain reaction template and used to amplify plasmid DNA as template (13). Similarly, the plasmid containing L5-RNA was constructed using the pHPIV3-CAT DNA and the primer 3 (5 ’-AAG CTT TAA TAC GAC CTA TAG TCA ATG TCT TTA ATC C-3’) containing a HindIII site, T7 promoter complementary sequence, and a 19-nucleotide complementary sequence from the leader region and primer 2 (5 ’-GTT ACC GAC GCT ATA TAC CAA AGA GAA GAA ACT TG-3’) containing KpnI-HgaI sites and 17 nucleotides from the untranslated region of the NP gene in a polymerase chain reaction. Sequence. Reactions of the inserts in these constructs were confirmed by DNA sequencing. Radiolabeled RNAs were synthesized using these plasmid DNAs after linearization with HpaI in an in vitro transcription reaction containing [α-32P]UTP and T7 RNA polymerase according to the manufacturer’s protocol (Boehringer Mannheim). The transcripts (73 nucleotides) would contain 55 nucleotides from the leader region and 18 nucleotides from the NP gene. The in vitro synthesized RNAs were analyzed in a 10% polyacrylamide-urea gel, and the radiolabeled RNA bands were excised. The RNAs were then eluted in a buffer containing 0.5 M ammonium acetate, 1 mM EDTA, and 0.1% SDS and purified by phenol/chloroform extraction. The purified RNA was electrophoresed on a 9% TBE (0.045 M Tris-borate/0.001 M EDTA) gel. The gel was run at 150 V at room temperature and then dried and subjected to autoradiography.

**UV Cross-linking**—Binding of purified cellular protein to the radiolabeled RNA was performed at room temperature for 30 min in the gel mobility shift assay buffer in a 96-well plate. The reaction mixture (20 μl) was then exposed to short wavelength UV light on ice at a 4-cm distance for 1 h. After UV cross-linking, the reaction mixture was incubated at 37°C for 5 min. The proteins were analyzed in a 10% SDS-polyacrylamide gel. The gel was stained, dried, and subjected to autoradiography.

**Sequence Analysis**—Proteins were resolved by electrophoresis in a 10% SDS-polyacrylamide gel and transferred onto polyvinylidene difluoride membrane according to the method of Matsudaira (14). The membrane was stained with Coomassie Blue, and the protein band was cut out, subjected to spot analysis on a PhosphorImager system model 470 sequencer equipped with an on-line phenylthiohydantoin analysis using the regular program 03RPTH.

**Immunoprecipitation of HPIV3 RNA**—Human lung carcinoma cells (A549) were grown in minimum Eagle’s medium and infected with HPIV3 at 10 PFU/cell. At 24 h postinfection, the cell lysate was prepared according to Horikami and Mayer (15). The cell lysate was used for immunoprecipitation of HPIV3 RNA with anti-GAPDH and anti-LA protein according to Chang et al. (9). The RNA was purified from the precipitated complex by phenol extraction and ethanol precipitation and was analyzed by RNA protection assay using radiolabeled 3’-GS- and LS-RNA probes according to Kurilla et al. (16).

**Purification of Intracellular RNP**—Intracellular RNP was isolated from HPIV3-infected CV-1 cells essentially as described by Toneguzzo and Ghosh (17), with slight modification. CV-1 cells in monolayer were infected with HPIV3 at 20 PFU/cell, and the cells were harvested at 20 h postinfection. The cells were washed with 10 mM phosphate buffer (pH 7.2) containing 150 mM NaCl and disrupted in 10 mM Tris-HCl (pH 7.8) by sonication. The cell lysate was centrifuged at 10,000 g for 10 min, and finally the RNP was purified from this supernatant by centrifugation at 100,000 g for 1 h.

**Immunofluorescent Labeling**—CV-1 cells were grown on coverslips and infected with HPIV3 at 1 PFU/cell. At 24 h postinfection, the cells were washed with phosphate-buffered saline followed by fixation with 3.6% paraformaldehyde and permeabilization with 1% Nonidet P-40. The fixed cells were treated with a mixture of rabbit anti-RNP and monoclonal anti-GAPDH or of rabbit anti-RNP and human anti-LA antibodies (18). For double labeling of RNP and GAPDH, the coverslips were washed and incubated with a mixture of fluorescein-conjugated anti-rabbit Ig and biotin-conjugated anti-mouse Ig secondary antibodies, followed by incubation with Texas Red-conjugated avidin. For double labeling of RNP and LA protein, the coverslips were washed and incubated with a mixture of fluorescein-conjugated anti-human Ig and biotin-conjugated anti-rabbit Ig secondary antibodies, followed by incubation with Texas Red-conjugated avidin. The coverslips were finally washed, mounted, and examined using a Leica CLSM confocal laser scanning microscope.

**RESULTS**

**Interaction of Cellular Proteins with HPIV3 cis-acting RNAs**—To identify cellular proteins that might be involved in HPIV3 gene expression, we inserted cDNA copies of the first 73 nucleotides from the 3’-end of the genome as well as its complementary sequence into transcription vectors under the control of T7 promoter to refer to as pUC3’-GS and pUCLS, respectively. Transcription of the plasmid pUC3’-GS by T7 RNA polymerase after linearization with HgoI gives rise to 3’-GS-RNA, while transcription from the plasmid pUCLS yielded the plus-sense LS-RNA (Fig. 1). The regulatory elements present within the 3’-GS-RNA include sites for the binding of viral RNA polymerase and putative cellular factors and also the intergenic trinucleotide GAA and NP gene start sequence that are believed to be involved in termination of the leader RNA and the initiation and capping of nascent RNA transcripts. The LS RNA, on the other hand, contains sites for initiation of encapsidation by NP and for interaction of viral and cellular proteins near the first intergenic region at which termination of RNA transcripts must be suppressed during replication. Radiolabeled 3’-GS- and LS-RNA probes were used in gel mobility shift assays with CV-1 cell cytoplasmic proteins. As shown in Fig. 3, no cellular two proteins were found that specifically interact with the LS RNA (complex I and II) and virtually one complex with the 3’-GS-RNA (complex I). The complexes with both 3’-GS- and LS-RNA probes were abolished in the presence of 40-fold
excess of corresponding unlabeled RNA, whereas 400-fold excess of unrelated competitor RNAs had no effect. In competition experiments with unlabeled heterologous RNA probes at 10-fold excess, complex I was significantly inhibited by both RNA probes, whereas 50-fold excess 3'-GS-RNA was required to inhibit the formation of complex II with LS RNA (data not shown). These results indicate that the formation of complex I most likely involves both sequence and structure of the RNA and that the same proteins are involved in interaction with the two RNA probes. Similar complexes were also formed when extracts were prepared from other cell lines such as human lung carcinoma (A549) and baby hamster kidney (data not shown), indicating the ubiquitous nature of the cellular proteins that formed complexes with the 3'-GS- and LS-RNAs of HPIV3. Next, we fractionated the CV-1 cell cytoplasmic extract using a DEAE-cellulose column where the complex I-forming activity was present in the unbound fraction and the complex II-forming activity was eluted from the column at around 0.4 M NaCl concentration (Fig. 2). Because the complex I- and II-forming activities were separable, we reasoned that two separate proteins were involved and set out to characterize the putative RNA-binding proteins.

**Glyceraldehyde-3-phosphate Dehydrogenase Is Involved in Forming Complex I**—To characterize the cellular protein that formed complex I, we first performed UV cross-linking analysis with the DEAE-cellulose unbound fraction. However, our attempt to identify the polypeptide directly by UV cross-linking failed, and accordingly we sought complete purification of the protein. The DEAE-cellulose unbound fraction was loaded onto a phosphocellulose column, and the bound proteins were eluted with a linear 0–1 M NaCl gradient. The complex I-forming activity was eluted around 0.5 M NaCl concentration (data not shown). The active fractions were pooled and subjected to further purification by successive chromatography on DEAE-cellulose and phosphocellulose columns. The complex I-forming protein was purified to near homogeneity, and molecular mass was estimated as ~37 kDa by SDS-polyacrylamide gel electrophoresis (Fig. 3A). For further characterization, we performed microsequence analysis of the protein and compared it with the protein sequences available in the database. As shown in Fig. 3, the partial sequence of the purified protein was virtually identical to the N terminus of bovine glyceraldehyde-3-phosphate dehydrogenase, a 37-kDa glycolytic enzyme (19). Consistent with these findings, the purified 37-kDa polypeptide...
reacted with a monoclonal anti-GAPDH antibody in Western blot analysis (Fig. 3B). Moreover, a commercial preparation of rabbit GAPDH (Boehringer Mannheim) also contained similar 3'-GS-RNA binding activity (Fig. 3C). Taken together, these data provide strong evidence that the complex I-forming protein is, in fact, GAPDH. Since GAPDH is not a bona fide RNA-binding protein but was shown to interact with poly(U) (20, 21), we investigated whether the stretches of U residues present in 3'-GS-RNA are involved in the interaction with GAPDH. As shown in Fig. 4A, the formation of complex I was inhibited by about 90% in the presence of 200-fold excess of poly(U), whereas a similar concentration of viral NP and P mRNAs had no effect, suggesting a role of U residues in this interaction. It is well documented that GAPDH contains a NAD$^+$ binding site that is conserved among dehydrogenases (22) and has also been shown to be involved in the binding of GAPDH to AU-rich RNA sequences (23). Therefore we tested whether this site is involved in the binding of 3'-GS-RNA. As shown in Fig. 4A, a high concentration of NAD$^+$ (>10 $\mu$M) was required to abolish 3'-GS-RNA binding activity, and other dehydrogenases such as glucose-6-phosphate dehydrogenase (G-6-PDH) and lactate dehydrogenase (LDH) (Boehringer Mannheim), P, phosphoprotein; NP, nucleocapsid protein.

### Polypeptide Pattern

Chromatographic separation of complex I- and II-forming proteins. The CV-1 cell cytoplasmic proteins (S100) were subjected to chromatography on a DEAE-cellulose column as described under "Experimental Procedures." Individual fractions (2 $\mu$l) were used in gel mobility shift assay with radiolabeled LS RNA. The complexes were analyzed by electrophoresis in 6% polyacrylamide gel. The gel was dried and subjected to autoradiography. The numbers at the top indicate the fraction eluted from DEAE-cellulose column, and $U$ represents the unbound fraction. The migration positions of complex I and II are indicated on the right.

### Specificity of Interaction

**Fig. 4. Specificity of interaction of GSBP with the 3'-GS-RNA.**

A, in gel mobility shift assay containing GSBP (100 ng) and radiolabeled 3'-GS-RNA (0.1 ng), 200-fold excess of unlabeled competitor RNAs as indicated (HPIV3 NP and P mRNAs were synthesized in vitro from pET3a-derived vectors) and 10 $\mu$M NAD$^+$ was added. B, gel mobility shift assay was performed with radiolabeled 3'-GS-RNA (0.1 ng) and 100 ng of the purified proteins, GSBP and commercial glucose-6-phosphate dehydrogenase (G-6-PDH) and lactate dehydrogenase (LDH) (Boehringer Mannheim). P, phosphoprotein; NP, nucleocapsid protein.

**Cellular Autoantigen, La, Is Involved in Forming Complex II**—To characterize the complex II-forming protein, we pooled the fractions containing LS RNA binding activity that eluted at around 0.4 M NaCl concentration from the DEAE-cellulose column (Fig. 2) and used them for further purification. The pooled fraction was subjected to chromatography on a Sephacryl S-200 column where the activity was eluted in a single peak (data not shown), and the purified protein was referred to as LSBP. As shown in Fig. 5A, the purified fraction contained several protein bands in a silver-stained SDS-polyacrylamide gel. To identify the polypeptide that is directly involved in the interaction with LS RNA, UV cross-linking was performed. As shown in Fig. 5A, an ~50-kDa polypeptide was cross-linked to the radiolabeled LS RNA indicating its involvement in the formation of complex II. Involvement of the same polypeptide in the formation of complex II with 3'-GS-RNA, albeit at a low level, was also confirmed by UV cross-linking with radiolabeled 3'-GS-RNA (data not shown). We speculated that the 50-kDa protein identified in our study might be the cellular LS protein since in previous studies specific interaction of cellular LA protein, a bona fide RNA-binding protein, was shown to interact with several viral RNAs (7–9, 24). To examine this possibility, we performed Western blot analysis of the purified LSBP with anti-LA antibody. As shown in Fig. 5B, the anti-LS antibody
specifically recognized the 50-kDa protein and control recombinant LA protein, strongly suggesting that LA protein is involved in the formation of complex II. To further confirm its identity, the radiolabeled LS RNA was incubated with purified LSBP, and the complex was immunoprecipitated with anti-LA antibody using protein A-Sepharose. As the control, we used polyclonal anti-actin antibody to immunoprecipitate the radiolabeled LS RNA. Finally, we used recombinant LA protein to study its ability to form complex II in a gel mobility shift assay using LS RNA. As shown in Fig. 5D, the radiolabeled LS RNA probe detected the viral RNA precipitated by anti-LA antibody, thus confirming the association of this cellular protein with HPIV3 RNA. Some precipitation of the genome-sense RNA by anti-actin antibody is observed that is possibly due to the association of actin with the RNP as a transcription factor (4). Next, we examined whether GAPDH remains associated with the viral RNP in HPIV3-infected cells. Intracellular RNP was isolated at different times postinfection, and the presence of GAPDH was determined by Western blot analysis. As shown in Fig. 6B, GAPDH was specifically associated with the viral RNP during infection. Similarly, in vivo association of LA protein with HPIV3 RNA was investigated by immunoprecipitation of viral RNA with anti-LA antibody and analysis of the precipitated RNA by RNase protection assay using radiolabeled 3'-GS-RNA probe. As shown in Fig. 7A, the radiolabeled 3'-GS-RNA probe detected the viral RNA precipitated by anti-LA antibody, thus confirming in vivo association of HPIV3 RNA with the cellular LA protein. We also examined whether the interaction of LA protein with the HPIV3 RNA leads to a specific association of this cellular protein with the viral RNP. The intracellular viral RNP was isolated, and the presence of LA protein was determined by Western blot analysis using anti-LA antibody. As shown in Fig. 7B, the LA protein was detected in the RNP as early as 8 h postinfection and continued to be present in the RNP during the virus life cycle.

Co-localization of GAPDH with HPIV3 RNP in the Infected Cells—Finally, to further confirm the interaction of viral RNP with these cellular proteins in the HPIV3-infected cells, indirect double immunofluorescence labeling and confocal microscopy were carried out. As illustrated in Fig. 8, GAPDH was
labeled predominantly in the cytoplasm of uninfected CV-1 cells with a perinuclear distribution (panel A). This distribution pattern of GAPDH remained unaltered in HPIV3-infected cells (panel B). An apparent increase in the level of GAPDH, following HPIV3 infection (compare panel A with B), is possibly due to formation of a multinucleated giant cell, which is typical of HPIV3 glycoprotein-mediated cell fusion. Interestingly, the viral RNP was also labeled in the cytoplasm with similar perinuclear distribution (panel C), suggesting specific interaction between the viral RNP and GAPDH. This notion was supported by the fact that, when confocal images were simultaneously acquired for both fluorochromes, the RNP and GAPDH were found to co-localize (panel D) in the HPIV3-infected cells. Immunolabeling of another cytoplasmic protein, tubulin, showed no co-localization with RNP, indicating that the interaction between the RNP and GAPDH was specific (data not shown). Similarly, we carried out double immunofluorescent labeling and confocal microscopy to examine specific interaction of viral RNP with the LA protein (data not shown). The LA protein was present primarily in the nucleus, and upon HPIV3 infection, a detectable amount was found to be redistributed in the cytoplasm in the perinuclear region where viral RNP was also present. However, co-localization as observed for GAPDH (panel D, yellow) could not be demonstrated due to low amounts of LA protein and the vast excess of RNP present in the same region. Nevertheless, these results strongly suggest that LA protein also becomes available for interaction with viral RNP in the cytoplasm.

**DISCUSSION**

In the present study, we have identified and characterized two cellular proteins, GAPDH, a cytoplasmic protein, and La, primarily a nuclear protein, that specifically interact with HPIV3 cis-acting regulatory RNAs, 3'GS- and LS-RNA, *in vitro* as well as *in vivo*. We have also demonstrated that the same two cellular proteins interact with viral RNP in HPIV3-infected cells. Moreover, by double immunofluorescent labeling and confocal microscopy, we have shown that GAPDH specifically co-localizes with viral RNP in the infected cells. The biological significance of these interactions, however, remain unknown at present. Nevertheless, both *in vitro* and *in vivo* specific interactions of GAPDH and LA protein with viral RNP strongly suggests that they must play a role in the life cycle of...
HPIV3. The involvement of GAPDH is particularly interesting and unexpected because it is not a bona fide RNA-binding protein, and its specific association with a virus has not been demonstrated before. It is primarily involved in cellular metabolism as the key enzyme of the glycolytic pathway. There are earlier reports where GAPDH has been implicated in binding to single-stranded RNA in polyribosomes (20, 25). Only recently a sequence-specific interaction of GAPDH with tRNAs (21) and AU-rich RNA sequences present in the 3′-untranslated region of several mRNAs has been reported (23). Since both 3′-GS- and LS-RNA contain AU-rich sequences, it is possible that GAPDH binds to these sequences. In addition, both cis-acting RNAs contain a similar stem-loop structure (Fig. 1) that could also be a part of the recognition site. The important question still remains with respect to the molecular basis of this selective interaction of GAPDH with HPIV3 RNA. Clearly, development of a reconstituted transcription or replication system in vitro using purified GAPDH would help delineate its role in these RNA synthetic processes.

It is important to note that GAPDH does not contain any consensus RNA binding motif similar to other well known RNA-binding proteins (26). In this regard, GAPDH appears to be similar to iron-responsive element binding protein (27), several small nuclear RNPs (28), and calreticulin (11), which bind to RNA but do not possess such RNA binding domains. The RNA binding properties of these proteins appear to be regulated by respective co-factors (21, 27) or by modification of the protein such as by phosphorylation (11). For GAPDH, the regulating co-factor is NAD+, and its binding site within GAPDH is commonly referred to as the Rossmann fold, which is conserved among dehydrogenases (22). Our findings that the interaction between 3′-GS-RNA and GAPDH is inhibited in vitro by NAD+, albeit at high concentration (10 μM) (Fig. 4A), suggest that the Rossmann fold may be involved in this interaction. Since other dehydrogenases such as lactate dehydrogenase and glucose-6-phosphate dehydrogenase did not bind 3′-GS-RNA (Fig. 4B), it suggests that the Rossmann fold may constitute only a part of the 3′-GS-RNA binding site in GAPDH. HPIV3 infection may also lead to a significant decrease in the intracellular concentration of NAD+, as observed recently in human immunodeficiency virus, type 1-infected cells (29) leading to inhibition of GAPDH activity with impairment of cellular functions. Thus, it remains to be determined whether HPIV3 may utilize some other activity of this cellular enzyme for its own replication while inhibiting glycolytic function of GAPDH. In this regard, it is particularly interesting to note that GAPDH also interacts with cellular actin (30), which has been shown to be involved in the activation of HPIV3 transcription (4). A detailed study along these lines would lead to better understanding of this unique host-virus interaction process.

The other cellular protein identified is the autoantigen LA protein, which binds specifically to the plus-sense leader RNA in vitro (Fig. 1). This protein also is found to be associated with HPIV3 RNP during infection suggesting again its possible role in virus replication. Cellular LA protein is a ubiquitously phosphoprotein and a bona fide RNA-binding protein found predominantly in the nucleus of cells (24, 31), and it was first identified as a target antigen of autoantibodies found in the sera of patients with systemic lupus erythematosus and Sjögren's syndrome (32). Interest in the LA protein was greatly stimulated by the finding that it binds to several RNA polymerase III transcripts (33) and facilitates their release from the template (34). Recently, cellular LA protein has also been shown to bind some viral RNAs such as adenovirus VA RNAs (35), Sindbis virus minus strand genome RNA (36), Epstein-Barr virus EBER RNAs (37), vesicular stomatitis virus and rabies virus leader RNAs (7, 8, 38), 5′-untranslated region of poliovirus RNA (24), and human immunodeficiency virus trans-activation response element RNA (9). Although the biological significance of the interaction between viral RNAs and cellular LA protein remained undefined, a specific role of LA protein in viral gene expression has more recently begun to emerge. For example, translation of poliovirus mRNA has been shown to require specific binding of LA protein to the 5′-untranslated region that relieves the structural constraint (39). Similarly, in the case of human immunodeficiency virus, the interaction of LA protein with the TAR element present at the 5′-end of the viral mRNAs was found to alleviate the translation repression by the TAR element (40). It is important to note that most of the viral RNAs reacting with LA are short, uncapped, and nonpolyadenylated, and the LA protein forms ribonucleoprotein complexes with these RNAs. Our studies also indicate that LA protein forms a ribonucleoprotein complex with the HPIV3 leader RNA because anti-LA antibody precipitated the La-bound leader RNA but not the free RNA. It is interesting to note that in the HPIV3 system the LA protein bound to leader RNA in vivo, which is elongated beyond the leader size (55 nucleotide) (Fig. 7). Leader length RNA (55 nucleotide), as found in VSV (7), was not detectable in HPIV3-infected cells raising the possibility that efficient elongation of RNA chains may occur once LA protein is bound to the nascent HPIV3 leader RNA. Thus, it would be interesting to determine whether LA protein binds at the intergenic region of the LS RNA. A consensus RNA motif for binding of LA protein has not been identified, and certain RNA sequences within the structural context, especially 3′-oligouribouridylicate sequence, are believed to be involved in this interaction (41). The HPIV3 plus-sense LS RNA does not contain long stretches of U sequences, however, internal di- and triuridylate repeats are noticeable. Moreover, as stated above, the secondary structure of LS RNA (Fig. 1) may also be involved in LA protein recognition. It should be noted that the 3′-GS-RNA, although containing U-rich sequences, does not interact with the LA protein (Fig. 1). Thus, the selective interaction with the LS RNA underscores an important role of LA protein in HPIV3 replication. Perhaps LA protein acts as an anti-terminator during the replicative process. Again, an in vitro transcription/replication system for HPIV3 will be needed to study the function of LA protein in the HPIV3 life cycle.

Finally, both GAPDH and LA protein appear to play a role in the life cycle of the virus not only due to their ability to bind to the cis-acting viral RNA sequences but also to the fact that they specifically associate with the RNP in the infected cells (Figs. 6 and 7), which is confirmed by double immunofluorescent labeling studies (Fig. 8). The co-localization of GAPDH and viral RNP demonstrates for the first time the possible involvement of a key metabolic enzyme in the life cycle of the virus. It remains to be seen at which steps of the virus replicative pathway GAPDH acts. The association of LA protein with RNP is interesting since it is essentially a nuclear protein, whereas HPIV3 replicates in the cytoplasm. However, by immunofluorescent studies (data not shown), a detectable amount of LA protein seems to be released in the cytoplasm (relative to uninfected control) following HPIV3 infection, as observed for poliovirus infection (24). Experiments are in progress to address the significance of these interactions by delineating the role of GAPDH and LA protein in HPIV3 gene expression.

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