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Specific Binding of Host Cell Proteins to the 3′-Terminal Stem-Loop Structure of Rubella Virus Negative-Strand RNA

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At the 5′ end of the rubella virus genomic RNA, there are sequences that can form a potentially stable stem-loop (SL) structure. The complementary negative-strand equivalent of the 5′-end SL structure of positive-strand rubella virus RNA [5′ (+) SL structure] is thought to serve as a promoter for the initiation of positive-strand synthesis. We screened the negative-strand equivalent of the 5′ (+) SL structure (64 nucleotides) and the adjacent region of the negative-strand RNA for their ability to bind to host cell proteins. Specific binding to the 64-nucleotide-long potential SL structure of three cytosolic proteins with relative molecular masses of 97, 79, and 56 kDa was observed by UV-induced covalent cross-linking. There was a significant increase in the binding of the 97-kDa protein from cells upon infection with rubella virus. Altering the SL structure by deleting sequences in either one of the two potential loops abolished the binding interaction. The 56-kDa protein also appeared to bind specifically to an SL derived from the 3′ end of positive-strand RNA. The 3′-terminal structure of rubella virus negative-strand RNA shared the same protein-binding activity with similar structures in alphaviruses, such as Sindbis virus and eastern equine encephalitis virus. A possible role for the host proteins in the replication of rubella virus and alphaviruses is discussed.

Rubella virus consists of a single-stranded polyadenylated genomic RNA of positive polarity, encapsidated by a capsid protein and contained within a lipid bilayer envelope in which the two virus-specific glycoproteins, E1 and E2, are embedded (9, 20, 27). In infected cells, a subgenomic RNA derived from the 3′ end of the genomic RNA is synthesized (20). The complete nucleotide sequence of the rubella virus genomic RNA has been recently determined, and it is 9,757 nucleotides long (4). The genomic RNA contains two long open reading frames, a 5′-proximal open reading frame of 6,615 nucleotides which encodes the nonstructural proteins and a 3′-proximal open reading frame of 3,189 nucleotides which encodes the structural proteins (4). The sequence of the 3′-terminal region of both the wild-type and the vaccine-type virus genomic RNA has been previously reported (3, 5, 6, 15, 17, 24, 26, 28).

Little is known about the replication of rubella virus. However, the organization of the genome of rubella virus is similar to that of alphaviruses and thus implies a similarity in the replication strategy (4, 16, 20). Sequence comparison of rubella virus genomic RNA with the alphavirus genomic RNA does not reveal significant homology (4). However, sequences similar to three regions of alphaviruses are found in the rubella virus genomic RNA (4). The three regions, which are highly conserved among alphaviruses, include sequences at the 5′ end of the genome, a 51-nucleotide conserved sequence near the 5′ end of the genomic RNA, and a 20-nucleotide conserved sequence at the junction of the genomic and subgenomic RNA (4). Functional analysis of the three conserved sequences in Sindbis virus RNA by Strauss and coworkers (10, 18, 19, 23) showed that these sequence elements are important for virus replication. Presumably, each performs a different function in virus replication (10, 18, 19). Previously, Schlesinger and colleagues (11, 12, 25) had arrived at a similar conclusion about the functions of the conserved sequences while studying Sindbis virus defective interfering RNA replication.

While the conserved sequences of Sindbis virus RNA were being analyzed, it became evident that they can fold into stem-loop (SL) structures (22). Because of the possible role of SL structures as protein recognition sites, we were intrigued by the observation made by Frey and coworkers (4) that the sequence at the 5′ end of the rubella virus genome can form a potential stable SL structure. Primer extension analysis of the 5′ end of the rubella virus genomic RNA revealed that there are strong stop bands both at the beginning of the potential rubella virus SL structure and at the 5′ end of genome RNA (4). Potential for formation of SL structures also has been observed at the 3′ end of rubella virus RNA (6, 17, 26, 28).

Understanding the mechanism of RNA virus replication is of prime significance because it is central to the pathogenicity of a large group of viruses. Despite its importance, very little is known about the details of this replication process in higher eukaryotic cells. Often, host factors are also required for RNA replication. The host factors may play a variety of roles in this process, including enzymatic, structural, and regulatory functions. In most cases, the host factors are not well characterized, nor are their functions well defined. Specific interaction of cellular proteins with RNA recognition elements might be one of the crucial steps involved in RNA replication. Such binding sites may be defined by a linear sequence or a particular sequence structure in the RNA. SL structures of RNA have been implicated to be important for translation, transcription, and replication. Specifically, the SL structure at the 5′ end of the poliovirus RNA has been shown to play a role in organizing viral and cellular proteins involved in positive-strand production (1). Similarly, it has been proposed that a cellular RNA-binding protein plays a role in mediating Tat-dependent long terminal repeat activation of human immunodeficiency virus (7).

Previously, we have shown that host proteins interact specifically with a potential SL structure found in the distal

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3′ end of the genomic RNA of rubella virus and that the increase in their binding activity after infection coincided temporally with the appearance of the negative-strand RNA synthesis (16). In this study, we examined the 3′-terminal SL structure of the negative-strand RNA [3′ (−) SL] for the ability to bind to protein(s) present in host cells.

MATERIALS AND METHODS

Viral infection of cells. Vero 76 cells were grown in T_{75} tissue culture flasks and infected with a plaque-purified stock of the wild-type rubella virus, M_{33} strain (5 PFU per cell). The viral titration and the period of infection were determined as described earlier (16).

Preparation of cell lysates. Cell lysates from both uninfected and infected cells were prepared according to the protocol described previously (16). Protein concentration was determined by the bicinchoninic acid assay (Pierce).

In vitro synthesis of RNA transcripts. Synthesis of oligonucleotide template along with the 17-base T7 promoter and its purification was performed as previously described (16). T7 polymerase was obtained from David Haile (Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development). Both labeled and unlabeled transcripts were generated as previously described (13). The oligonucleotide sequence for the template of the rubella virus 3′ (−) SL RNA structure was 5′-ACCTCGCTTAGG ACTCCCATTCCCATGGAGAAACTCCTAGATGAGGT CCTATAGTGAGTCGTATTA-3′; for Sindbis virus the template was 5′-GATTGGCGGCGTAGTACACACTATTGAA TCAAAACAACCGACCAATTGCACTACCCTATAGTGA GTCGTATTA-3′; and for eastern equine encephalitis virus the template was 5′-GATAGGGTATGGTGTAGAGGCAG CCACCCGACCTATCCTATCCTATAGTGAGTCGTAT TA-3′. The RNA for the 3′-end SL of the rubella virus positive-strand RNA [3′ (+) SL] was synthesized as described previously (16). The iron response element (IRE) probe was synthesized as described in reference 21. Poly(I · C) was purchased from Boehringer Mannheim Biochemicals, Inc.

Gel retardation assays. Gel retardation assays were performed by using 500 to 700 pmol of ^{32}P-labeled RNA probe (approximately 20,000 cpm) and 20 μg of protein in 20-μl volumes containing cytolysis buffer and RNase inhibitor (Inhibit-ACE; 5 Prime-3 Prime, Inc., Boulder, Colo.) (8). Binding was performed at room temperature for 20 min. The complexes were resolved on a 6% polyacrylamide nondenaturing gel for 1.5 h with Tris-borate-EDTA buffer as previously described (21). After electrophoresis, the gels were fixed and dried.

In vitro UV-induced cross-linking and SDS-PAGE analysis of cross-linked proteins. Cell lysates (20 μg) from uninfected and infected cells were incubated with 700 pmol of a high-specific-activity SL RNA probe (70,000 cpm/ng) for 30 min at 4°C. RNA-protein complexes were cross-linked at room temperature in a water bath with a UV Stratalinker 2400 (Stratagene Co.) for 30 min at 1,200 μJ × 100. Samples were then treated with RNase T_1 (1 U) for 10 min at room temperature. Samples were boiled in Laemmli sample buffer and analyzed by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (17).

RESULTS

Identification of cellular proteins that bind to SL structure at 3′ end of rubella virus negative-strand RNA. To look for the proteins which might interact with the 3′ SL of negative-strand RNA, we synthesized an RNA molecule encompassing the 64 bases of the possible SL structure (Fig. 1A). The radiolabeled RNA was used as a probe to search for RNA-binding proteins by the use of the gel mobility shift assay and UV cross-linking technique from both uninfected and infected cytosols (Fig. 2). Using lysates from infected and uninfected cells with several RNA probes as competitors, we showed that the two band-shift complexes (I and II) observed are specific for the 3′ (−) SL RNA (Fig. 2A). When the proteins from the lysates were UV cross-linked in the presence of the RNA probe and resolved by SDS-PAGE, two protein bands of 56 and 79 kDa were observed from

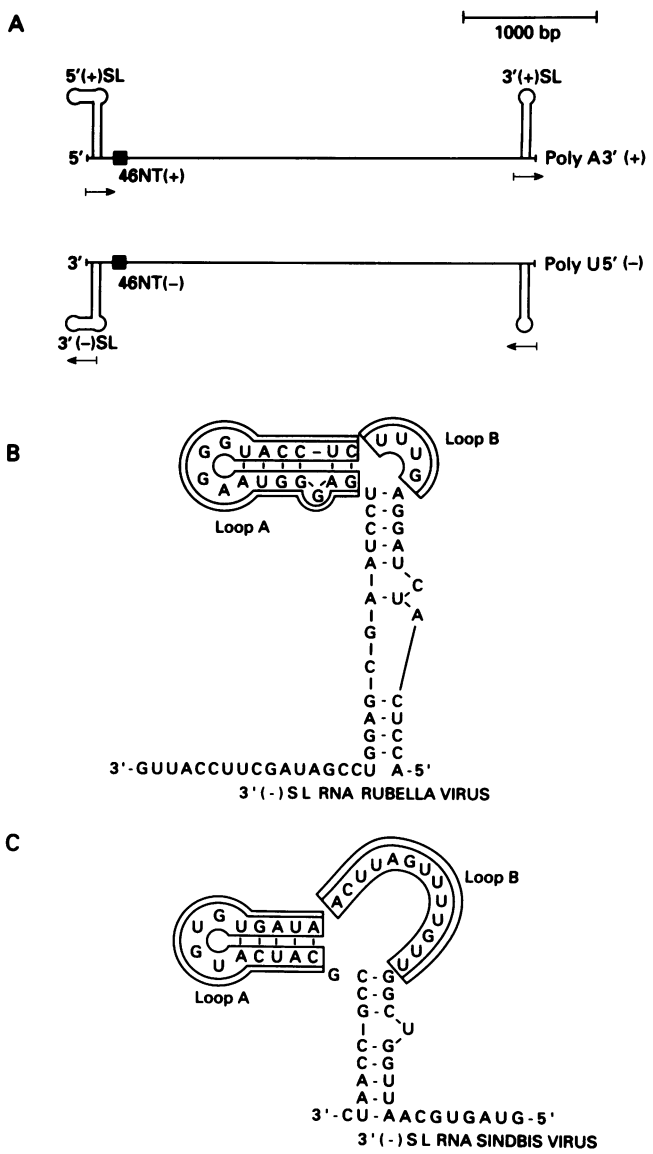

FIG. 1. (A) Schematic representation of rubella virus positive (+)- and negative (−)-strand RNAs; 5′ (+) SL, 5′-end SL structure of the positive strand; 3′ (+) SL, 3′-end SL structure of the positive strand; 3′ (−) SL, 3′-end SL structure of the negative strand; 46NT(+) or (−), 46-nucleotide conserved sequence of either the positive- or the negative-strand rubella virus RNA. (B) Proposed SL structure of the 3′ (−) SL of rubella virus RNA. (C) Proposed SL structure of the 3′ (−) SL of Sindbis virus RNA.

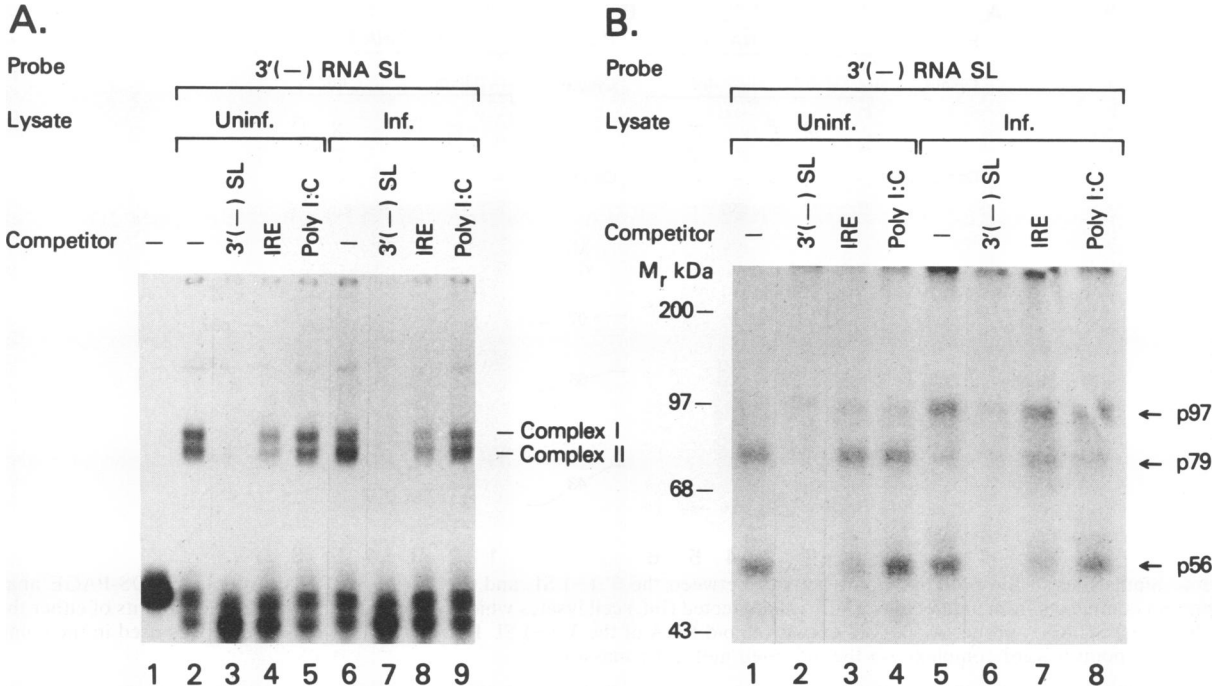

FIG. 2. (A) Gel retardation assay of the rubella virus 3′ (−) SL RNA. Lysates (20 μg) from uninfected (Uninf.) and infected (Inf.) cells were incubated with 700 pmol of RNA probe alone or in the presence of a 500-fold excess of specific [3′ (−) SL] or nonspecific [IRE or poly(I · C)] RNA. (B) SDS-PAGE analysis of the RNA-protein complexes formed by UV cross-linking with the 3′ (−) SL probe. The amount of cell lysate and the quantity of the probe is the same as in panel A. The RNA samples used for the competition are shown. Arrows point to complexes at the indicated molecular masses.

uninfected lysates (Fig. 2B, lane 1), whereas an additional protein band of 97 kDa was observed from infected lysates (Fig. 2B, lane 5). The weak binding seen at 97 kDa in the uninfected extracts was not greater than the nonspecific background (Fig. 2B, lanes 1 to 4). An excess unlabeled RNA of identical sequence and polarity could specifically block the binding activity (Fig. 2B, lanes 2 and 6), whereas unrelated RNAs, such as an IRE or poly(I · C), did not block the activity (Fig. 2B, lanes 3, 4, 7, and 8). The slight competition of p56 between the labeled rubella virus probe and the IRE probe is not significant as was evidenced by quantitating the bands by scanning densitometry. Furthermore, when molar ratios of specific and nonspecific competitors to the probe were decreased, the specificity was readily apparent and the IRE no longer showed any effect. The specificity of the binding activity was confirmed by the absence of competition in the presence of an RNA probe upstream of the SL structure (see Fig. 7A and B, lanes 5).

To address the specificity of the interactions, we made several variants of the SL RNA of rubella virus (Fig. 1B). The results of cross-linking experiments are shown in Fig. 3. Computer-based modeling (29) predicts that the 3′ SL of the negative-strand RNA can form a long stem with two loops designated A and B (Fig. 1B). When sequences which can potentially form either one of the two loops were deleted, there was drastic reduction in the protein-binding activity from both uninfected and infected cell lysates (Fig. 3, lanes 3 to 8). The weak binding of cellular proteins to the truncated RNA probes was not greater than the nonspecific background (Fig. 3, lanes 3 to 8). However, altering the sequences in the possible stem region so that the base-paired structure was no longer present did not change the binding activity (data not shown). The intensity of the protein-RNA complexes observed both in the gel shift assay and in UV-induced cross-linking varied from experiment to experiment.

Similarities in binding activities between 3′ SL structures of negative-strand and positive-strand rubella virus RNA. We

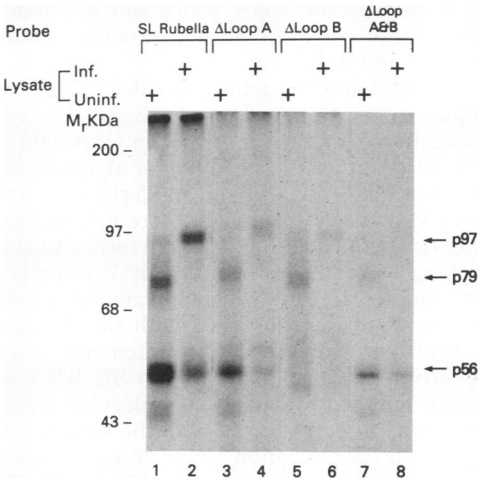

FIG. 3. Effect of mutations in the 3′ (−) SL RNA of rubella virus on the RNA-protein interaction. SDS-PAGE analysis of RNA-protein complexes from uninfected (Uninf.) and infected (Inf.) cell lysates which were incubated with equal amounts of RNA probes. SL Rubella, 3′ (−) SL RNA; Δ loop A, deletion of nucleotides 32 to 48; Δ loop B, deletion of nucleotides 49 to 52; Δ loops A and B, deletion of nucleotides 32 to 52. Arrows point to complexes at the indicated molecular masses.

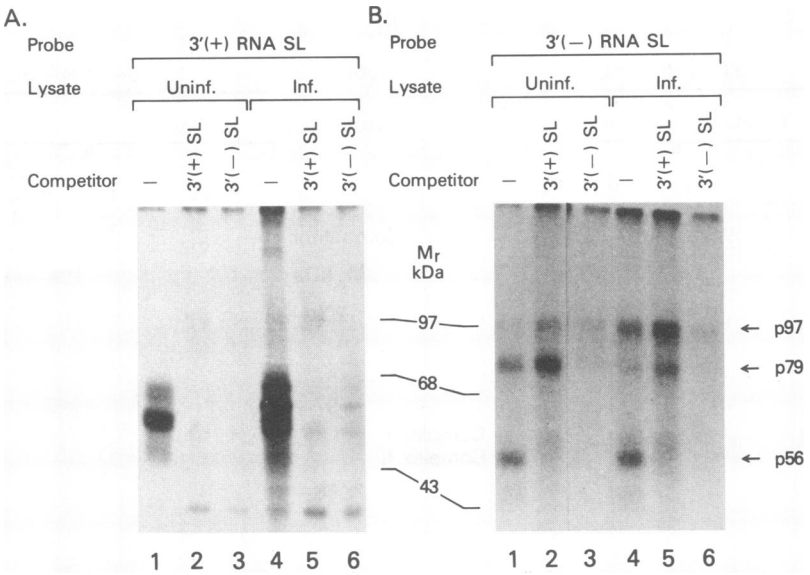

FIG. 4. Similarities in the protein-binding activity between the 3′ (−) SL and the 3′ (+) SL RNA structures. SDS-PAGE analysis of RNA-protein complexes from uninfected (Uninf.) and infected (Inf.) cell lysates which were incubated with equal amounts of either the 3′ (−) SL or the 3′ (+) SL RNA probe. A 100-fold excess of cold RNA of the 3′ (−) SL RNA or the 3′ (+) SL RNA was used in the competition assay. Arrows point toward complexes at the indicated molecular masses.

determined whether the binding activity of the 3′ SL of negative-strand RNA [3′ (−) SL] has any common component(s) with the binding activity of the 3′ SL of positive-strand RNA [3′ (+) SL] that we have previously studied and characterized (16). As shown in Fig. 4A, lanes 3 and 6, the binding activity of the 3′ (+) SL RNA from uninfected and infected cell lysates was blocked by an excess of 3′ (−) SL RNA. However, the reverse was not true. When cold 3′ (+) SL RNA was used for competition, only the binding activity of 56-kDa protein was blocked (Fig. 4B, lanes 2 and 5). p79 and p97 binding to the 3′ (−) SL probe was enhanced when 3′ (+) SL RNA was added to the reaction (Fig. 4B, lanes 2 and 5). A nonspecific RNA probe did not function as a competitor for 3′ (−) RNA SL complex formation (Fig. 2A, lanes 4, 5, 8, and 9).

3′ (−) SL RNA-binding activity of rubella virus is shared by other alphaviruses. Comparison of the possible 3′ SL structure of negative-strand RNA of rubella virus with the potential 3′ SL of negative-strand RNAs of alphaviruses such as Sindbis virus showed striking similarities in the overall secondary structure even though there is little homology in the nucleotide sequence (Fig. 1C). We turned to UV-induced cross-linking of cellular proteins with 3′ (−) SL RNAs to examine whether SL RNAs from alphaviruses have binding activity similar to that observed with rubella virus. Using cell lysates from uninfected and infected cells, we observed proteins cross-linked with the 3′ (−) SL RNA of Sindbis virus RNA that were similar in molecular weight to the proteins observed cross-linked with the rubella virus 3′ (−) SL RNA, with the exception of the 56-kDa protein (Fig. 5, compare lanes 9 and 13 with lanes 1 and 5). The RNA-protein complexes with rubella virus and Sindbis virus 3′ (−) SL RNA were blocked by their homologous and heterologous 3′ (−) SL RNAs (Fig. 5, lanes 2, 3, 6, 7, 10, 11, 14, and 15). Similar results were observed when the 3′ (−) SL of eastern equine encephalitis virus RNA was used in the competition assay (Fig. 5, lanes 4, 8, 12, and 16). The specificity of the RNA-protein interaction with the Sindbis virus and eastern equine encephalitis virus 3′ (−) SL RNA was demonstrated by lack of competition in the presence of nonspecific RNAs (data not shown). To further confirm that similar protein-RNA complexes are recognized by both rubella virus and Sindbis virus SL structures, we made variants of Sindbis virus 3′ (−) SL RNA. The results of the cross-linking experiment showed that deletion of sequences which can potentially form loop B resulted in significant reduction of the binding activity of 97- and 79-kDa proteins (Fig. 6, lanes 3 and 4). The effect was not so prominent with p56 binding; possibly, there is a lower rate of binding in this experiment. We observed variability in binding of p56 in many other experiments as well.

Interaction of 46-nucleotide conserved sequence with binding activity of 3′ (−) SL RNA. It was observed (4) that there is a stretch of 46 nucleotides located 224 nucleotides from the 5′ end of the rubella virus genome (Fig. 1A) which has 50% overall homology with the 51-nucleotide conserved sequence of alphaviruses. The 51-nucleotide sequence of Sindbis virus has been implicated in viral replication (19). We therefore asked whether the 46-nucleotide region in the rubella virus RNA plays a role in viral replication similar to that of the 3′ (−) SL RNA via RNA-protein interaction. The results (Fig. 7) showed that the negative strand of the 46-nucleotide conserved sequence of rubella virus blocked the binding activity of the 3′ (−) SL RNA (Fig. 7A, lane 4) and that the same was true when the 3′ (−) SL RNA was used as a competitor in the binding assay for the negative strand of the 46-nucleotide conserved sequence (Fig. 7B, lane 4). However, the 51-nucleotide conserved sequence of negative-strand RNA of Sindbis virus did not compete with this interaction except for the 56-kDa protein (Fig. 7A and B, lanes 6). Quantitation of the p56 band by scanning densitometry revealed that the competition by the 51-nucleotide sequence from Sindbis virus is significant. To prove the specificity of this interaction, we found that RNA from the adjoining region of the 46-nucleotide sequence did not compete with the activity (Fig. 7A and B, lanes 5).

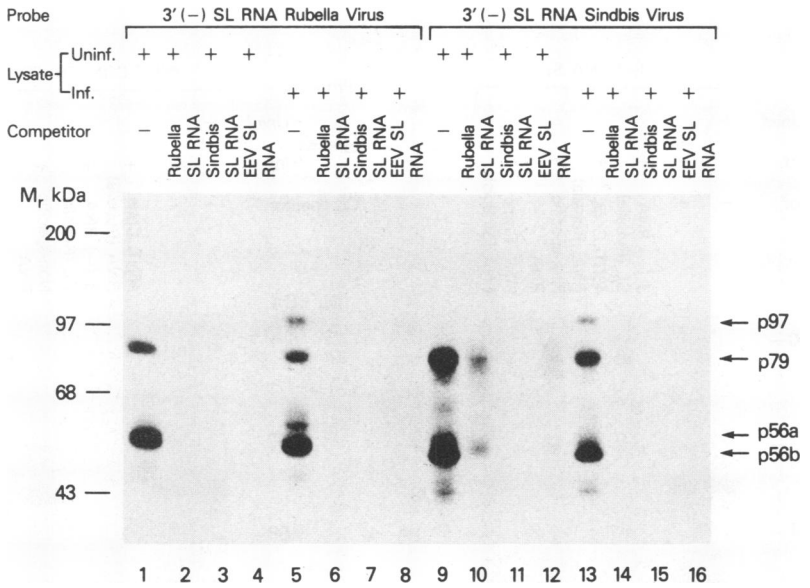

FIG. 5. Similarities in the protein-binding activity between the 3′ (−) SL RNAs of rubella virus and alphaviruses (Sindbis virus and eastern equine encephalitis virus [EEV]). SDS-PAGE analysis of RNA-protein complexes formed in the presence or absence of unlabeled 3′ (−) SL RNAs of rubella virus, Sindbis virus, and eastern equine encephalitis virus with equal amounts of probes. The molecular masses of the complexes are shown. Uninf., uninfected; Inf., infected.

DISCUSSION

The rubella virus genomic RNA is predicted to form potentially stable SL structures at both the 5′ and the 3′ ends (4, 6, 28). These secondary structures in the RNA have been implicated in viral replication (4). Similar structures in the Sindbis virus RNA have been shown to be important for the replication of Sindbis virus and its defective interfering particles (10, 12, 18, 19).

Previously, we have shown that host cell proteins interact specifically with the possible 3′ (+) SL of the genomic RNA of rubella virus and that the increase in protein-binding activity coincided with the appearance of negative-strand RNA synthesis (16). In this study, we demonstrated the existence of another group of cellular proteins which interact specifically with the potential 3′-end SL structure of the negative-strand rubella virus RNA [3′ (−) SL]. The interaction of these proteins is specific as demonstrated by the lack of inhibition by unrelated RNA. Mutational analysis of the 3′ (−) SL structure showed that the sequences which can form two potential loop structures are involved in the protein-binding activity (Fig. 3). The cellular protein interaction with the 3′ (−) SL RNA suggests an involvement of these proteins in the replication process. Recently, we were able to demonstrate the initiation of negative-strand RNA synthesis from a chloramphenicol acetyltransferase RNA which had rubella virus 5′- and 3′-end possible SL structures (14). The initiation of negative-strand RNA synthesis was achieved only after viral infection (14). Mutations in the first 44 nucleotides at the 5′ end of the Sindbis virus RNA, which are capable of forming an SL structure, showed that this region has a role to play in viral replication since some of the mutations were either lethal or resulted in poor viral growth in different cell types (19). This led these investigators to suggest that there is an interaction of host factors with the Sindbis virus SL structures.

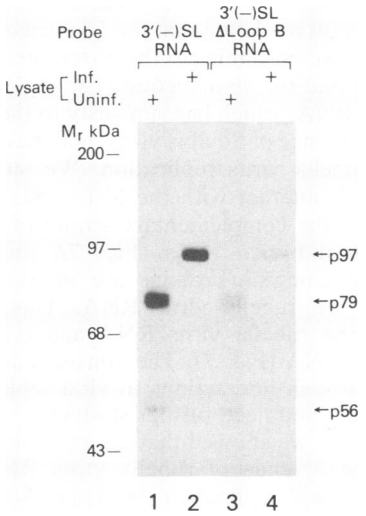

FIG. 6. Effect of mutations in the 3′ (−) SL RNA of Sindbis virus on the protein-RNA interaction. SDS-PAGE analysis of RNA-protein complexes from uninfected (Uninf.) and infected (Inf.) cell lysates. Δ loop B, nucleotides 25 to 37 were deleted in the 3′ (−) SL RNA. Arrows depict the molecular masses of the complexes.

While analyzing the interaction of the cellular proteins with the possible 3′ (−) SL structure, we realized that one of the proteins, i.e., the 56-kDa protein, has a molecular mass (61 kDa) approximately similar to that of the 3′ (+) SL RNA-binding protein from uninfected cells (16). We therefore reasoned that the two proteins may be the same. Competition analysis of the binding activity between the two SL structures showed that indeed the two proteins recognize similar structures. Recognition of the 56-kDa protein by both the positive-strand and negative-strand SL RNAs suggests that this protein is involved in two processes, one to initiate the negative-strand synthesis and the other to initiate the positive-strand synthesis. Occurrence of multiple nonidentical RNA-binding domains in a single protein has been

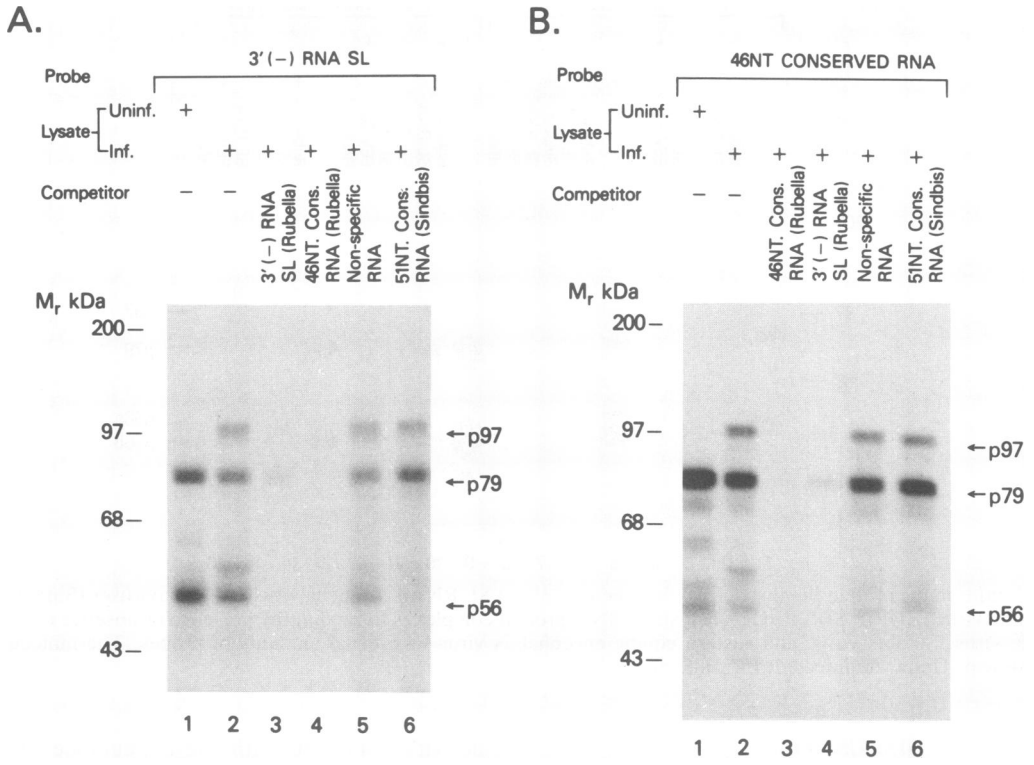

FIG. 7. Involvement of the 46-nucleotide conserved sequence (46NT. Cons.) in the 3′ (−) SL RNA-protein interaction. (A) SDS-PAGE analysis of RNA-protein complexes from uninfected (Uninf.) and infected (Inf.) cell lysates with the 3′ (−) SL RNA. The competition assay was done in the presence of a 500-fold excess of specific and nonspecific RNAs. (B) SDS-PAGE analysis of RNA-protein complexes from uninfected and infected cell lysates with the negative strand of the 46-nucleotide conserved sequence. The designation of each lane is indicated at the top. Arrows point toward the molecular masses of the complexes. 51NT Cons., 51-nucleotide conserved sequence.

observed with several RNA-binding proteins, and these distinct domains have been postulated to have several different functions (2). The discrepancy in the molecular masses of the 3′ (−) SL RNA-binding protein (56 kDa) reported in the present study and the 3′ (+) SL RNA-binding protein (61 kDa) reported earlier (16) will be the subject of further study.

Based on the observation (4) that the 5′ (+) SLs of rubella virus and Sindbis virus have similar stable RNA secondary structures, we reasoned that the 3′ (−) SL RNA of Sindbis virus binds to proteins similar to those which interact with the 3′ (−) SL of rubella virus RNA. Indeed, the two potential negative-strand SL structures compete with each other for the same cellular binding proteins, and the similarity in the binding activity is also extended to another member of the alphavirus family, eastern equine encephalitis virus. Reduction in the binding activities with the mutant forms of both the Sindbis virus and rubella virus 3′ (−) SL RNA structures further suggests that the binding interaction between the two viruses involves similar structures. However, it remains to be seen whether exchange of the 5′ (+) SL structures between the Sindbis virus and the rubella virus RNAs can allow initiation of replication from either of the viral genomes.

In the rubella virus genomic RNA, within 224 nucleotides from the 5′ end, there is a stretch of 46 nucleotides which has 50% overall similarity with the Sindbis virus 51-nucleotide conserved sequence (4). This region of the Sindbis virus, which is conserved among all alphaviruses, is located 155 nucleotides from the 5′ end and is capable of forming double-hairpin structures in a computer-based model (22). The function of this element is not clear, but it has been implicated in viral replication (19). Secondary structure analysis of the first 250 bases of Sindbis virus RNA by these investigators (19) predicted that the 51-nucleotide conserved region is in close proximity to the 5′-terminal SL structure. We reasoned that the 46-nucleotide conserved region in the rubella virus RNA, which has similarity to the 51-nucleotide conserved sequence of Sindbis virus, may have a similar role to play in rubella virus replication. We showed that the proteins which interact with the 3′ (−) SL RNA are also recognized by the complementary strand of the 46-nucleotide element and vice versa (Fig. 7A and B), thereby implying that common proteins are interacting with two elements of the rubella virus RNA. This interaction is specific for the rubella virus RNA and is different from Sindbis virus RNA (Fig. 7). The consequences of this and other RNA-protein interactions in viral replication are not clear at present and need further studying.

In conclusion, we showed that the possible SL structures at the 5′ and 3′ ends of rubella virus RNA, which are necessary for viral replication, bind specifically to cellular proteins. There is a common protein which interacts with both elements. Similarity in the binding activity of the 3′-end elements of the negative-strand RNA of rubella virus and alphaviruses implies that common factors are involved in their respective replication processes.

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REFERENCES

1. **Andino, R., G. E. Rieckhof, and D. Baltimore.** 1990. A functional ribonucleoprotein complex forms around the 5′ end of poliovirus RNA. Cell **63**:369–380.
2. **Bandziuilis, R. J., M. S. Swanson, and G. Dreyfuss.** 1989. RNA-binding proteins as developmental regulators. Genes Dev. **3**:431–437.
3. **Clark, D. M., T. W. Loo, I. Hui, P. Chong, and S. Gillam.** 1987. Nucleotide sequence and in vitro expression of rubella virus 24S subgenomic messenger RNA encoding the structural proteins E1, E2 and C. Nucleic Acids Res. **15**:3041–3057.
4. **Dominguez, G., C.-Y. Wang, and T. K. Frey.** 1990. Sequence of the genome RNA of rubella virus: evidence for genetic rearrangement during togavirus evolution. Virology **177**:225–238.
5. **Frey, T. K., and L. D. Marr.** 1988. Sequence of the region coding for virion protein C and E2 and the carboxy terminus of the nonstructural proteins of rubella virus: comparison with alphaviruses. Gene **62**:85–99.
6. **Frey, T. K., L. D. Marr, M. L. Hemphill, and G. Dominguez.** 1986. Molecular cloning and sequence of the region of the rubella virus genome coding for glycoprotein E1. Virology **154**:228–232.
7. **Gatignol, A., A. Buckler-White, B. Berkhout, and K.-T. Jeang.** 1991. Characterization of a human TAR RNA binding protein that activates the HIV-1 LTR. Science **251**:1597–1660.
8. **Hentze, M. W., T. A. Rouault, J. B. Harford, and R. D. Klausner.** 1989. Oxidation-reduction as a molecular mechanism of a regulatory RNA-protein interaction. Science **244**:357–359.
9. **Ho-Terry, L., and A. Cohen.** 1982. Rubella virion polypeptides: characterization by polyacrylamide gel electrophoresis, isoelectric focusing and peptide mapping. Arch. Virol. **72**:47–54.
10. **Kuhn, R. J., Z. Hong, and J. H. Strauss.** 1990. Mutagenesis of the 3′ nontranslated region of Sindbis virus RNA. J. Virol. **64**:1465–1476.
11. **Levis, R., H. Haung, and S. Schlesinger.** 1987. Engineered defective interfering RNAs of Sindbis virus express bacterial chloramphenicol acetyltransferase in avian cells. Proc. Natl. Acad. Sci. USA **84**:4811–4815.
12. **Levis, R., B. G. Weiss, M. Tsiang, H. V. Haung, and S. Schlesinger.** 1986. Deletion mapping of Sindbis virus DI RNAs derived from cDNAs defines the sequences essential for replication and packaging. Cell **44**:137–145.
13. **Milligan, J. F., D. R. Groebe, G. W. Witherell, and O. C. Uhlenbeck.** 1987. Oligoribonucleotide synthesis using T7 RNA polymerase and synthetic DNA templates. Nucleic Acids Res. **15**:8783–8798.
14. **Nakhasi, H. L., X.-Q. Cao, T. A. Rouault, and R. D. Klausner.** Unpublished data.
15. **Nakhasi, H. L., B. C. Meyer, and T.-Y. Liu.** 1986. Rubella virus cDNA: sequence and expression of E1 envelope protein. J. Biol. Chem. **261**:16616–16621.
16. **Nakhasi, H. L., T. A. Rouault, D. J. Haile, T.-Y. Liu, and R. D. Klausner.** 1990. Specific high-affinity binding of host cell proteins to the 3′ region of rubella virus RNA. New Biol. **2**:255–264.
17. **Nakhasi, H. L., D. Thomas, D. Zheng, and T.-Y. Liu.** 1989. Nucleotide sequence of capsid, E2 and E1 protein genes of rubella virus vaccine strain RA27/3. Nucleic Acids Res. **17**:4393–4394.
18. **Niesters, H. G. M., and J. H. Strauss.** 1990. Defined mutation in the 5′ end nontranslated sequence of Sindbis virus RNA. J. Virol. **64**:4162–4168.
19. **Niesters, H. G. M., and J. H. Strauss.** 1990. Mutagenesis of the conserved 51-nucleotide region of Sindbis virus. J. Virol. **64**:1639–1647.
20. **Oker-Blom, C., I. Ulmanen, L. Kaariainen, and R. F. Pettersson.** 1984. Rubella virus 40S genomic RNA specifies a 24S subgenomic mRNA that codes for a precursor to structural proteins. J. Virol. **49**:403–408.
21. **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.
22. **Strauss, E. G., and J. H. Strauss.** 1986. Structure and replication of the alphavirus genome, p. 35–90. *In* S. Schlesinger and M. J. Schlesinger (ed.), The togaviridae and flaviviridae. Plenum Publishing Corp., New York.
23. **Strauss, J. H., and E. G. Strauss.** 1988. Evolution of RNA viruses. Annu. Rev. Microbiol. **42**:657–683.
24. **Takkinen, K., G. Vidgren, J. E. Ulf-Hellman, N. Kalkkinen, C. Wrenstedt, and R. F. Pettersson.** 1988. Nucleotide sequence of the rubella virus capsid protein gene reveals an unusually high G/C content. J. Gen. Virol. **69**:603–612.
25. **Tsiang, M., B. G. Weiss, and S. Schlesinger.** 1988. Effects of 5′ terminal modification on the biological activity of defective interfering RNAs of Sindbis virus. J. Virol. **62**:47–53.
26. **Vidgren, G., K. Takkinen, N. Kalkkinen, L. Kaarianen, and R. F. Pettersson.** 1987. Nucleotide sequence of the genes coding for the membrane glycoproteins E1 and E2 of rubella virus. J. Gen. Virol. **68**:2347–2357.
27. **Waxham, M. N., and J. S. Wolinsky.** 1983. Immunochemical identification of rubella virus hemagglutinin. Virology **126**:194–203.
28. **Zheng, D., L. Dickens, T.-Y. Liu, and H. L. Nakhasi.** 1989. Nucleotide sequence of the 24S subgenomic messenger RNA of a vaccine strain (HPV77) of rubella virus: comparison with a wild type strain (M33). Gene **82**:343–349.
29. **Zuker, M., and P. Stiegler.** 1981. Optimal computer folding of large RNA sequences using thermodynamics and auxiliary information. Nucleic Acids Res. **9**:133–148.