Hantaviruses, members of the Bunyaviridae family, are negative-stranded emerging RNA viruses and category A pathogens that cause serious illness when transmitted to humans through aerosolized excreta of infected rodent hosts. Hantaviruses have evolved a novel translation initiation mechanism, operated by nucleocapsid protein (N), which preferentially facilitates the translation of viral mRNAs. N binds to the ribosomal protein S19 (RPS19), a structural component of the 40S ribosomal subunit. In addition, N also binds to both the viral mRNA 5′ cap and a highly conserved triplet repeat sequence of the viral mRNA 5′ UTR. The simultaneous binding of N at both the terminal cap and the 5′ UTR favors ribosome loading on viral transcripts during translation initiation. We characterized the binding between N and RPS19 and demonstrate the role of the N-RPS19 interaction in N-mediated translation initiation mechanism. We show that N specifically binds to RPS19 with high affinity and a binding stoichiometry of 1:1. The N-RPS19 interaction is an enthalpy-driven process. RPS19 undergoes a conformational change after binding to N. Using T7 RNA polymerase, we synthesized the hantavirus S segment mRNA, which matches the transcript generated by the viral RNA-dependent RNA polymerase in cells. We show that the N-RPS19 interaction plays a critical role in the translation of this mRNA both in cells and rabbit reticulocyte lysates. Our results demonstrate that the N-mediated translation initiation mechanism, which lures the host translation machinery for the preferential translation of viral transcripts, primarily depends on the N-RPS19 interaction. We suggest that the N-RPS19 interaction is a novel target to shut down the N-mediated translation strategy and hence virus replication in cells.

Hantaviruses, members of the Bunyaviridae family, are category A pathogens and causative agents of two emerging diseases: hemorrhagic fever with renal syndrome and hantavirus cardiopulmonary syndrome with mortalities of 15 and 50%, respectively (1, 2). Hantaviruses are transmitted to humans through aerosolized excreta of infected rodent hosts. The spherical hantavirus particles harbor three negative sense genomic RNA segments (S, L, and M) within a lipid bilayer (3). The mRNAs derived from S, L, and M segments encode viral nucleocapsid protein (N),2 viral RNA-dependent RNA polymerase (RdRp), and glycoprotein precursor, respectively. The glycoprotein precursor is cleaved at a conserved WAASA site, and two glycoproteins, Gn and Gc, are generated (4). The characteristic feature of the hantaviral genome is the partially complementary sequence at the 5′ and 3′ termini of each of the three genome segments that undergo base pairing and form panhandle structures (5–7). N is a multifunctional protein playing vital roles in multiple processes of the virus replication cycle and enters the host cell along with viral capsid during infection. N has been found to undergo trimerization both in vivo and in vitro (8–20). During encapsidation, N specifically recognizes the three viral RNA genome segments inside the host cell and targets them for packaging. Multiple in vitro studies have shown that N preferentially binds viral RNA compared with complementary RNA or nonviral RNA (14, 21–26). It has been proposed that the specific binding of N with either the panhandle or the sequence at the 5′ terminus alone selectively facilitates the encapsidation of viral RNA to generate three nucleocapsids that are packaged into infectious virions (26, 27). The RNA binding domain of Hantaan virus N has been mapped to the central conserved region corresponding to amino acid residues 175–217(25).

The nucleocapsids composed of viral RNA and N are used as templates by viral RdRp for genome replication and mRNA synthesis (28, 29). The RdRp from segmented negative sense RNA viruses requires a capped RNA oligo as a primer to initiate the transcription (30–34). The capped RNA primer is generated from the 5′ terminus of host cell mRNA by the unique “cap snatching” mechanism that has been well characterized in influenza virus. Although the knowledge about the sequence, length, and structure of the 5′ mRNA terminus that donates the primer is rather limited, most common cap donor mRNAs are cleaved 15 nucleotides downstream of the 5′ cap with a variation of 10–20 nucleotides (31, 32, 35–41). Exceptions have been reported for the members of the Arenaviridae family and Nairovirus genus that use relatively shorter primers, varying in length from one to four and from five to 16 nucleotides, respec-

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2 The abbreviations used are: N, nucleocapsid protein; RPS19, ribosomal protein S19; RdRp, RNA-dependent RNA polymerase; 1,5-IAEDANS, 5-(((2-io-dacetyl)amino)ethyl)amino)naphthalene-1-sulfonic acid; bis-ANS, 4,4′-bis(1-anilinonaphthalene-8-sulfonate); Ni-NTA, nickel-nitrilotriacetic acid; SNV, Sin Nombre virus.
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We have recently reported that hantavirus N binds to the 5' caps of host cell mRNAs and protects their degradation from the 5' terminus by the cellular mRNA degradation machinery (15). The rescued 5' capped mRNA oligos are used as primers by viral RdRp during transcription initiation (15). The capped RNA primers remain attached with the 5' terminus of viral mRNAs, which improves their stability and promotes their ability to serve as better templates for protein synthesis by the host cell translation machinery.

All viral mRNAs are translated by the host cell translation machinery. Viral mRNAs have to compete with the cellular transcripts for the same cellular translation apparatus. Viruses avoid such competition by using unique translation strategies that hijack the cellular translation machinery for the preferential translation of viral mRNAs. Such viral translation strategies are mainly driven by cis-acting higher order structures in the viral mRNA, such as internal ribosome entry sites (44–46) and ribosome shunt elements (47), which selectively load ribosomes on viral mRNAs. In addition, viruses have evolved unique mechanisms that modify host cell translation factors and shut down the translation of host cell mRNAs, making the translation machinery abundantly available for the translation of viral transcripts (48).

We have recently reported that hantviruses initiate the translation of their mRNAs by a unique mechanism operated by viral N (17). N binds to the mRNA 5' caps and 40 S ribosomal subunits and preferentially loads them on capped mRNAs, thereby augmenting their translation (17). The 40 S ribosomal subunit is a huge complex of 32 ribosomal proteins and 18 S rRNA. We have recently reported that N binds to the ribosomal protein S19 (RPS19), a structural component of the 40 S ribosomal subunit (49). However, one would like to know the role of this translation mechanism in the translation of viral mRNAs. Hantaviral mRNAs contain a short 5' cap and a highly conserved triplet repeat sequence (UAGUAGUAG). We recently reported that monomeric and trimeric N molecules specifically bind to the 5' cap and a highly conserved triplet repeat sequence of the viral mRNA 5' UTR, respectively (38, 42, 43).

In comparison with nonviral transcripts that lack the viral 5' cap, viral N (17) binds to the mRNA 5' terminus by the cellular mRNA degradation machinery. The rescued 5' capped mRNA oligos are used as primers by the viral RdRp during transcription initiation (15). The capped RNA primers remain attached with the 5' terminus of viral mRNAs, which improves their stability and promotes their ability to serve as better templates for protein synthesis by the host cell translation machinery.

Experimental Procedures

Oligonucleotides and Enzymes—PCR primers were from Integrated DNA Technologies. All restriction enzymes were from New England Biolabs. Anti-RPS19 antibody was from Santa Cruz Biotechnology. Phusion DNA polymerase was from Finnzymes. DNase I, T7 transcription reagents, rabbit reticulocyte lysates, cap analog, and RNase inhibitor were from Promega. DNA and RNA purification reagents were from Qiagen. The fluorophore 1,5-IAEDANS was from Invitrogen. All other chemicals, including bis-ANS, were obtained from Sigma. Radioactive [35S]methionine was from PerkinElmer Life Sciences.

Constructs—The gene encoding N was derived from Sin Nombre hantavirus strain 77734 (51, 52) and cloned in pTriEx 1.1 vector (Novagen) to generate the expression vector pSNV N as described previously (17). Another construct, pSNV N UTR was generated for the expression of S segment mRNA that contained appropriate 5' and 3' UTR sequences. The expression construct was driven by T7 RNA polymerase. An appropriately positioned hepatitis delta virus ribozyme sequence was incorporated in the plasmid to generate the 3' minus in the newly synthesized mRNA that matches exactly with the 3'-end generated by the hantaviral RdRp. This plasmid was constructed as follows. First, a T7 terminator was excised from pSNV N plasmid using NotI and Sphi restriction sites and cloned between the same sites in a pUC19 vector to generate the pUC19 plasmid. Second, the gene encoding the viral S segment mRNA with appropriate 5' and 3' UTR sequences was PCR-amplified from pADS vector (27) using the forward primer (5'-ATATATTCGGTTAATACGACCTATATAGAAGTGATTAGTATAGACTCCTTGGAACTAGCTAC-TACGACTAAAG) and the reverse primer (5'-ATATATTAGCCGCCCTCTCATATTATACCCCTGTGTAATAATATTGGGCCCTG). The forward primer with flanking T7 promoter contained the Xmal restriction site, and the reverse primer contained the NotI and BsmBI restriction sites. The PCR product was digested with XmaI and NotI and incorporated between the same sites in pUC17ter to generate the plasmid pTSNVNT7ter. Third, the hepatitis delta virus ribozyme sequence was PCR-amplified using the forward primer (5'-ATATATTTATTACGTCCTCATATAGAAGTGATTAGTATAGACTCCTTGGAACTAGCTAC-TACGACTAAAG) and the reverse primer (5'-ATATATTAGCCGCCCTCTCATATTATACCCCTGTGTAATAATATTGGGCCCTG). The forward primer contained the BsmBI restriction site, and the reverse primer contained the Xhol restriction site. The PCR product was digested with these enzymes and incorporated between the same sites in pUC17ter to generate the plasmid pTSNVNT7ter.

Viral mRNAs are translated by the host cell translation machinery. Viral mRNAs have to compete with the cellular transcripts for the same cellular translation apparatus. Viruses avoid such competition by using unique translation strategies that hijack the cellular translation machinery for the preferential translation of viral mRNAs. Such viral translation strategies are mainly driven by cis-acting higher order structures in the viral mRNA, such as internal ribosome entry sites (44–46) and ribosome shunt elements (47), which selectively load ribosomes on viral mRNAs. In addition, viruses have evolved unique mechanisms that modify host cell translation factors and shut down the translation of host cell mRNAs, making the translation machinery abundantly available for the translation of viral transcripts (48).

We have recently reported that hantviruses initiate the translation of their mRNAs by a unique mechanism operated by viral N (17). N binds to the mRNA 5' caps and 40 S ribosomal subunits and preferentially loads them on capped mRNAs, thereby augmenting their translation (17). The 40 S ribosomal subunit is a huge complex of 32 ribosomal proteins and 18 S rRNA. We have recently reported that N binds to the ribosomal protein S19 (RPS19), a structural component of the 40 S ribosomal subunit (49). However, one would like to know the role of this translation mechanism in the translation of viral mRNAs. Hantaviral mRNAs contain a short 5' cap and a highly conserved triplet repeat sequence (UAGUAGUAG). We recently reported that monomeric and trimeric N molecules specifically bind to the 5' cap and a highly conserved triplet repeat sequence of the viral mRNA 5' UTR, respectively (38, 42, 43). Using competitive translation assays, we found that N requires the viral mRNA 5' UTR to favor the translation of viral transcripts in comparison with nonviral transcripts that lack the viral 5' UTR sequence (50). Here, we characterized the interaction between N and RPS19. Our results suggest that the N-RPS19 interaction likely facilitates ribosome loading on capped mRNA during the N-mediated translation initiation mechanism. Thus, the N-RPS19 interaction is a novel target for the development of inhibitors to shut down N-mediated translation initiation and inhibit hantavirus replication in cells.

Experimental Procedures

Oligonucleotides and Enzymes—PCR primers were from Integrated DNA Technologies. All restriction enzymes were from New England Biolabs. Anti-RPS19 antibody was from Santa Cruz Biotechnology. Phusion DNA polymerase was from Finnzymes. DNase I, T7 transcription reagents, rabbit reticulocyte lysates, cap analog, and RNase inhibitor were from Promega. DNA and RNA purification reagents were from Qiagen. The fluorophore 1,5-IAEDANS was from Invitrogen. All other chemicals, including bis-ANS, were obtained from Sigma. Radioactive [35S]methionine was from PerkinElmer Life Sciences.

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Similarly, another plasmid, pLUC UTR, was constructed by replacing the N open reading frame (ORF) from pSNV UTR with firefly luciferase ORF. The pLUC UTR plasmid was used to transfect BSRT7-5 cells for luciferase expression. However, the luciferase mRNA generated by T7 RNA polymerase from this construct contained luciferase ORF sandwiched between 5' and 3' UTRs of the viral S segment mRNA. The expression plasmid pRPS19 was constructed for the expression of RPS19 in bacteria and mammalian cells. The total RNA purified from HeLa cells using an RNaseq kit (Qiagen) was reverse transcribed using a reverse primer (5'-ATCTAACTCGAGCTAA-TGGCTTCTTGGTGCG) specific for RPS19 mRNA. The reverse transcription product was PCR-amplified using a forward primer (5'-ATGTATCCCATGGTGCCTGGAGTTAC-TGCTTCTTGTTGGCAG) and the above reverse primer. The PCR product was digested with NcoI and XhoI and cloned in pTriEx 1.1 vector between the same restriction sites. The plasmid pRPS19mut, which expresses an RPS19 mutant containing
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a cysteine residue at the C terminus prior to His6 tag, was generated using the same strategy except the reverse primer contained an extra codon for the cysteine residue upstream of the XhoI restriction site.

Expression and Purification of Hantavirus N and RPS19—The Rosetta DE3 pLacI bacterial cells (Novagen) were transformed with pTSNVN plasmid for the expression of N, which contained a six-histidine tag at the C terminus for purification on Ni-NTA beads, as described previously (14, 17). The same bacterial cells were transformed with either pRPS19 or pRPS19mut plasmid for the expression of wild type or mutant RPS19 proteins, respectively. Bacterial cultures grown in 1-liter flasks were induced with isopropyl 1-thio-β-d-galactopyranoside for protein expression. We observed very high expression for both wild type and mutant RPS19 proteins in these bacterial cells. Both proteins were purified on Ni-NTA beads using a standard purification protocol provided by the manufacturer (Qiagen).

Labeling of RPS19 Mutant with 1,5-IAEDANS—The 1,5-IAEDANS fluorescent label was dissolved in DMSO, and peak absorption at 336 nm was recorded using spectrophotometer. Concentration of the stock solution was determined using an extinction coefficient of 5700 M⁻¹ cm⁻¹ (Molecular Probes). The RPS19 mutant at a concentration of 50 μM in buffer A (40 mM Tris, pH 7.5, 100 mM NaCl, 1 mM EDTA) and 1,5-IAEDANS fluorescent label were mixed at a molar ratio of 1:40 and incubated at 30 °C for 2 h and then at 4 °C overnight in the dark. The unbound 1,5-IAEDANS label was removed by dialysis against buffer A.

Immunoprecipitation Analysis—Immunoprecipitation analysis was carried out as described previously (49). Briefly, Protein G-agarose beads (20-μl packed volume) were washed with radioimmune precipitation assay buffer (50 mM Tris, pH 7.5, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.05% SDS, 1 mM EDTA, 150 mM NaCl) containing protease inhibitor mixture and RNasin (1 unit/50 μl). Washed beads were incubated with either anti-RPS19 or anti-N polyclonal antibody or rabbit serum at 4 °C for 2 h with gentle rotation. The bacterially expressed and purified N at a concentration of 200 nM was mixed with either wild type or mutant RPS19 labeled with 1,5-IAEDANS at a molar ratio of 1:1 in a total reaction volume of 100 μl. The mixture was incubated at room temperature for 1 h followed by further incubation with antibody-coated beads at 4 °C overnight. The beads were collected after centrifugation at 3000 rpm for 2 min and washed five times with radioimmune precipitation assay buffer. Washed beads were resuspended in elution buffer (50 mM Tris, pH 7.5, 5 mM EDTA, 10 mM DTT, 1% SDS) and centrifuged at 3000 rpm for 5 min, and the supernatant was analyzed for the presence of either N or RPS19 by Western blot analysis using the appropriate antibody.

Fluorescence Spectroscopic Studies—Binding of 1,5-IAEDANS-labeled RPS19 with N was carried out in a Shimadzu spectrofluorometer (RF-5301PC). The fluorescence spectrum of 1,5-IAEDANS-labeled RPS19 protein from 400 to 600 nm was recorded in binding buffer (50 mM Tris, pH 7.4, 80 mM NaCl, 20 mM KCl) using an excitation wavelength of 336 nm (excitation slit width, 5 nm; emission slit width, 10 nm). The steady state value of fluorescence emission at 485 nm with time indicated the absence of photodegradation of 1,5-IAEDANS-labeled RPS19. All fluorescence binding experiments were carried out at room temperature except the experiments used for the calculation of the thermodynamic parameters of the N-RPS19 interaction. To a fixed concentration of 1,5-IAEDANS-labeled RPS19 (200 nM), the concentration of N was gradually increased, and the fluorescence spectrum of 1,5-IAEDANS (400–600 nm) was recorded at each input concentration of N. For calculating the dissociation constant (Kd), the fluorescence value at 485 nm was recorded at each input concentration of N. The binding profile was generated by plotting the fluorescence value at 485 nm along the y axis and the N concentration along the x axis.

The dissociation constant (Kd) for the N-RPS19 interaction was calculated by nonlinear curve fitting analysis of the data points based on the following equilibrium: L + P ⇄ LP where L and P represent N and 1,5-IAEDANS-labeled RPS19, respectively. Curve fitting was carried out according to Equations 1 and 2 (54, 55). Goodness of the fit was ascertained by least square analysis.

\[
K_d = (C_p - (\Delta F/\Delta F_{max})C_p)(C_i - (\Delta F/\Delta F_{max})C_i)/((\Delta F/\Delta F_{max})C_p)
\]  
(Eq. 1)

\[
C_p(\Delta F/\Delta F_{max})^2 - (C_p + C_i + K_d)(\Delta F/\Delta F_{max}) + C_i = 0
\]  
(Eq. 2)

\[\Delta F = 1/\Delta F_{max} + K_q/(\Delta F_{max}(C_i)) \]

(Eq. 3)

The Kq values calculated from three independent experiments were used for the calculation of standard deviation.

To monitor the conformational changes in RPS19 due to the binding of N, we monitored the quenching of 1,5-IAEDANS fluorescence of RPS19 by a neutral quencher (acrylamide) before and after the binding of N according to the Stern-Volmer equation (57) \[F_0/F = 1 + K_{sv}[Q]\] where \(F_0\) and \(F\) are the fluorescence intensities in the absence and presence of the quencher respectively, \([Q]\) is the concentration of the quencher, and \(K_{sv}\) is the Stern-Volmer quenching constant. Small aliquots of acrylamide from a concentrated stock solution were added to 300 μl of 1,5-IAEDANS-labeled RPS19 at a concentration of 100 mM in binding buffer, and the fluorescence signal of 1,5-IAEDANS at 485 nm was recorded at each input concentration of acrylamide. Similar quenching studies were carried out with 1,5-IAEDANS-labeled RPS19 that was preincubated with 500 mM N. The data points in a plot of \(F_0/F\) versus \([Q]\) fit to a straight line, indicating a single species of 1,5-IAEDANS moieties that are equally accessible to the neutral quencher. The Stern-Volmer quenching constant (\(K_{sv}\)) was calculated from the slope of the straight line fitted to the data points as reported previously (14, 58). The \(K_{sv}\) values calculated from three independent experiments were used for the calculation of standard deviation.
Fluorescence studies of hydrophobic fluorophore bis-ANS were carried out in a Shimadzu spectrofluorometer (RF-5301PC). The fluorophore was dissolved in dimethyl sulfoxide, and its concentration was determined from the extinction coefficient ($\epsilon_{390} = 24,000 \text{ m}^{-1} \text{ cm}^{-1}$). The fluorophore was excited at 399 nm, and emission spectra were recorded at room temperature in binding buffer from 420 to 600 nm. To a fixed concentration of either wild type RPS19 or 1,5-IAEDANS-labeled RPS19 (100 nM), small aliquots of bis-ANS were added from a higher concentration stock, and the fluorescence value at 485 nm was recorded at each input concentration. Because the excitation wavelength for 1,5-IAEDANS fluorophore is 336 nm and the fluorescence emission wavelength range is similar to bis-ANS (400–600 nm), we confirmed that excitation of 1,5-IAEDANS-labeled RPS19 at 399 nm does not show any emission at 485 nm. To determine the change in fluorescence signal of bis-ANS due to binding with RPS19, the fluorescence signal of free bis-ANS in binding buffer without RPS19 was subtracted.

**Competition Experiments**—We carried out competition experiments to confirm the specific interaction between N and RPS19. To a fixed concentration of 1,5-IAEDANS-labeled RPS19 (100 nM) and N (500 nM), the concentration of unlabeled RPS19 was gradually increased, and the fluorescence signal at 485 nm was recorded. The fluorescence intensity at 485 nm was plotted versus input concentration of the unlabeled RPS19, and data points were fit by nonlinear curve fit analysis with the Origin 6 program (MicroCal). The inhibition constant, $IC_{50}$, corresponds to the concentration of unlabeled RPS19 required to obtain half-saturation, which is consistent with 50% inhibition in the interaction between N and 1,5-IAEDANS-labeled RPS19. The $IC_{50}$ values calculated from three independent experiments were used for the calculation of standard deviation.

**Calculation of Binding Stoichiometry**—As described previously (14, 59), the binding stoichiometry (expressed in terms of the number of RPS19 molecules bound per molecule of N) was estimated from the intersection of two straight lines of a least square fit plot of $\Delta F/\Delta F_{\text{max}}$ against the ratio of input concentrations of N and 1,5-IAEDANS-labeled RPS19. We also used continuous variation plots to verify the binding stoichiometry results as reported previously (14, 59, 60). This is a reliable method to determine the binding stoichiometry of protein–protein complexes. At a constant temperature (25 °C), the fluorescence signal was recorded for the solutions where the concentrations of both 1,5-IAEDANS-labeled RPS19 and N were varied, keeping the sum of their concentrations constant at 340 nm. $\Delta F_{485}$ (the difference in the fluorescence intensity of 1,5-IAEDANS-labeled RPS19 in the absence or presence of N) was plotted as a function of the input mole fraction of RPS19. The breakpoint in the resulting plot corresponds to the mole fraction of RPS19 in the N–RPS19 complex. The binding stoichiometry is obtained in terms of RPS19:N ($\chi_{\text{ligand}}^{-1} = \chi_{\text{ligand}}$, where $\chi_{\text{ligand}}$ represents the molar concentration of RPS19 divided by the total molar concentration of RPS19 and N).

**Analysis of Thermodynamic Parameters**—Thermodynamic parameters $\Delta H$ (van’t Hoff enthalpy), $\Delta S$ (entropy), and $\Delta G$ (free energy) for the association of 1,5-IAEDANS-labeled RPS19 with N were calculated with Equations 4 and 5,

$$\ln K_{\text{app}} = -\Delta H/RT + \Delta S/R$$  
(Eq 4)

$$\Delta G = \Delta H - T\Delta S$$  
(Eq 5)

where $R$ and $T$ are the universal gas constant and absolute temperature, respectively. The $K_{\text{app}}$ (apparent equilibrium constant $= 1/K_D$) was calculated at four different temperatures (8, 15, 25, and 30 °C) by fluorometric titration of 1,5-IAEDANS-labeled RPS19 with N. $\Delta H$ and $\Delta S$ were calculated from the slope and intercept of a plot of $\ln K_{\text{app}}$ against $1/T$ (Equation 4). $\Delta G$ was calculated from Equation 5 after incorporation of the values for $\Delta H$ and $\Delta S$ obtained from Equation 4. The experiment was repeated three times for the calculations of standard deviations in thermodynamic parameters.

**T7 Transcription for mRNA Synthesis**—The viral S segment mRNA containing appropriate 5' and 3' UTRs was synthesized by a T7 transcription reaction using the RiboMax RNA production kit (Promega). The gene encoding viral S segment mRNA was PCR-amplified from pSNV UTR plasmid using two opposing primers. The PCR product was gel-purified and used as template for mRNA synthesis in a T7 transcription reaction. The transcription reactions were performed following the manufacturer's protocol (Promega). The mRNA generated was purified using an RNAeasy kit (Qiagen) and used for translation in rabbit reticulocyte lysates as described below.

**Translation Assays in Rabbit Reticulocyte Lysates**—Nuclelease-treated rabbit reticulocyte lysates were used for the translation of mRNA in the presence and absence of either bacterially expressed and purified N, RPS19, or both. We synthesized the viral S segment mRNA with appropriate 5' and 3' UTRs by a T7 transcription reaction as described under “T7 Transcription for mRNA Synthesis.” One microgram of this mRNA was translated in a 50-μl reaction containing 35 μl of rabbit reticulocyte lysate, 1 μl of an amino acid mixture lacking methionine (1 mM), 1 μl of [35S]methionine (1175 Ci/mmol), 2 μl of RNase inhibitor (40 mg/ml), 4 μl of mRNA in water (250 ng/μl), and 7 μl of RNA-free water. Reaction mixtures were incubated at 30 °C for 20 min. Samples were electrophoresed on 10% SDS gels and quantified with a phosphorimaging system. We observed that S segment mRNA was poorly translated in rabbit reticulocyte lysates. To determine whether N helps the translation of its own mRNA, we added purified N to the translation reaction at a final concentration of 100 nM. To delineate whether the N-RPS19 interaction has a role in N-mediated translation initiation, we carried out translation reactions containing a fixed concentration of N (100 nM) with increasing concentrations (100–500 nM) of purified RPS19. As a control, translation reactions containing the same increasing concentrations of purified RPS19 without N were also carried out.

**Luciferase Assay**—The BSRT7-5 cells were cotransfected with pLUC UTR construct along with either pSNV N, pRPS19, or both and lysed 24 h post-transfection. Cells were lysed with luciferase cell lysis buffer (Promega), and luciferase assays were performed according to the manufacturer’s protocol.
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RESULTS

Expression and Purification of RPS19 and SNV N—The genes encoding RPS19 and SNV N were cloned in pTriEx 1.1 vector as described under “Experimental Procedures.” *Escherichia coli* cells were transformed with the expression vectors, and colonies were screened for the expression of C-terminally His-tagged RPS19 and SNV N by Western blot using either anti-RPS19 or anti-N antibody. *E. coli* cells harboring either RPS19 or SNV N expression vectors were grown in 1-liter cultures and induced with isopropyl 1-thio-β-D-galactopyranoside for RPS19 and N expression, respectively. Both proteins were purified using Ni-NTA column as described under “Experimental Procedures.” SDS-PAGE analysis showed that both the proteins were free of contaminating bacterial proteins (Fig. 1A). The electrophoretic mobility of N and RPS19 was consistent with a molecular mass of 52 and 17 kDa, respectively, as evidenced by comigration relative to that of protein molecular mass markers. We previously reported the interaction between N and RPS19, and here we wanted to quantitatively estimate the binding affinity and further characterize the interaction between N and RPS19, and hence the function of RPS19.

Characterization of N-RPS19 Interaction—We used fluorescence spectroscopy to further characterize the interaction between N and RPS19. The 1.5-IAEDANS-labeled RPS19 was excited at 336 nm, and the emission spectrum from 400 to 600 nm was recorded. The fluorescence spectrum of 1.5-IAEDANS bound to RPS19 showed an emission peak at 485 nm (Fig. 2A). However, the fluorescence spectrum of free 1.5-IAEDANS
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showed an emission peak at 488 nm (not shown). The 3-nm blue shift in the 1,5-IAEDANS fluorescence emission peak after covalent attachment to the cysteine side chain is due to shielding of the fluorescent moiety by RPS19. We observed a consistent decrease in the fluorescence quantum yield of 1,5-IAEDANS-labeled RPS19 with a 9-nm blue shift in the emission peak at saturating concentrations of N (Fig. 2A). The fluorescence signal was 50% quenched with a 9-nm blue shift in the emission peak at saturating concentrations of N (Fig. 2A). The decrease in the fluorescence quantum yield and blue shift in the emission spectra are due to the interactions between N and RPS19. We did not notice any change in the fluorescence spectrum of 1,5-IAEDANS-labeled RPS19 at similar increasing concentrations of BSA, consistent with specific interaction between N and RPS19. A complex of N and 1,5-IAEDANS-labeled RPS19 resulted in the quenching of the 1,5-IAEDANS fluorescence signal (Fig. 2A). As expected, the complex formation between N and 1,5-IAEDANS-labeled RPS19 for binding to N. A complex of N and 1,5-IAEDANS-labeled RPS19 was titrated with unlabeled RPS19, and the fluorescence signal at 485 nm was recorded. The fluorescence intensity at 485 nm was plotted versus input concentration of the unlabeled RPS19. The inhibition constant, IC50, was calculated as described under “Experimental Procedures.”

To determine the pH dependence of the binding reaction, we performed competition experiments in which unlabeled RPS19 competed with 1,5-IAEDANS-labeled RPS19 for binding to N. A complex of N and 1,5-IAEDANS-labeled RPS19 was titrated with unlabeled RPS19, and the fluorescence signal at 485 nm was recorded. As expected, the complex formation between N and 1,5-IAEDANS-labeled RPS19 resulted in the quenching of the 1,5-IAEDANS fluorescence signal (Fig. 2D). However, we observed a consistent increase in the fluorescence signal with increasing input concentrations of unlabeled RPS19, indicating that unlabeled RPS19 competes with 1,5-IAEDANS-labeled RPS19 for binding to N (Fig. 2D). Analysis of the fluorescence competition data revealed an IC50 value of 89 ± 2 nM, which is consistent with a Kd value of 95 ± 3 nM for the N-RPS19 interaction.

interaction between N and RPS19, we carried out competition experiments in which unlabeled RPS19 competed with 1,5-IAEDANS-labeled RPS19 for binding to N. A complex of N and 1,5-IAEDANS-labeled RPS19 was titrated with unlabeled RPS19, and the fluorescence signal at 485 nm was recorded. As expected, the complex formation between N and 1,5-IAEDANS-labeled RPS19 resulted in the quenching of the 1,5-IAEDANS fluorescence signal (Fig. 2D). However, we observed a consistent increase in the fluorescence signal with increasing input concentrations of unlabeled RPS19, indicating that unlabeled RPS19 competes with 1,5-IAEDANS-labeled RPS19 for binding to N (Fig. 2D). Analysis of the fluorescence competition data revealed an IC50 value of 89 ± 2 nM, which is consistent with a Kd value of 95 ± 3 nM for the N-RPS19 interaction.

To determine the pH dependence of the binding reaction, we performed the binding experiments in Tris buffer at different pH values. This analysis showed that N binds to RPS19 with high affinity at pH 7.5 (Fig. 3A).

FIGURE 2. Fluorescence spectroscopic analysis for interaction of N with 1,5-IAEDANS-labeled RPS19. A, as described under “Experimental Procedures,” a fixed concentration of 1,5-IAEDANS-labeled RPS19 (200 nM) in binding buffer was excited at 336 nm, and the fluorescence spectrum from 400 to 600 nm was recorded (black line). The experiment was carried out at 25 °C. The fluorescence signal from free binding buffer in the absence of RPS19 was subtracted wherever required. The fluorescence spectra of 1,5-IAEDANS-labeled RPS19 at increasing concentrations of purified N (shown by arrows) are presented in different colors. The input concentration of N (nM) corresponding to each spectrum is shown by arrows. B, the fluorescence intensity of 1,5-IAEDANS-labeled RPS19 at 485 nm was recorded at each input concentration of N, and a plot of fluorescence intensity (at 485 nm) as a function of N concentration is shown. C, the data from B were used to generate a plot of ΔF/ΔFmax versus N concentration for the calculation of Kd using Equations 1 and 2 as described under “Experimental Procedures.” The Kd values calculated from three independent experiments were used for the calculation of standard deviation (shown in Table 1). D, a competition experiment to check the specificity of the N-RPS19 interaction. To a fixed concentration of 1,5-IAEDANS-labeled RPS19 (100 nM) and N (500 nM), the concentration of unlabeled RPS19 was gradually increased (0–750 nM), and fluorescence signal at 485 nm was recorded. The 3-nm shift in the emission peak at saturating concentrations of N (Fig. 2A) was used to generate the binding profile (Fig. 2C) for the calculation of the dissociation constant (Kd) as described under “Experimental Procedures.” This analysis revealed that N interacts with RPS19 with a dissociation constant of 95 ± 3 nM. To check the specificity of the interaction between N and RPS19, we carried out the binding reactions at three different NaCl concentrations. As shown in Table 1, we did not observe a noticeable change in Kd values at different salt concentrations, consistent with the specific interaction between N and RPS19. Similar results were obtained when reactions were carried out in three different buffers at different salt concentrations (Table 1). To further confirm the specific

The fluorescence signal was 50% quenched with a 9-nm blue shift in the emission peak after covalent attachment to the cysteine side chain is due to shielding of the fluorescent moiety by RPS19. We observed a consistent decrease in the fluorescence quantum yield of 1,5-IAEDANS-labeled RPS19 with a 9-nm blue shift in the emission peak at saturating concentrations of N (Fig. 2A). The decrease in the fluorescence quantum yield and blue shift in the emission spectra are due to the interactions between N and RPS19. We did not notice any change in the fluorescence spectrum of 1,5-IAEDANS-labeled RPS19 at similar increasing concentrations of BSA, consistent with specific interaction between N and RPS19. A complex of N and 1,5-IAEDANS-labeled RPS19 resulted in the quenching of the 1,5-IAEDANS fluorescence signal (Fig. 2A).

As expected, the complex formation between N and 1,5-IAEDANS-labeled RPS19 for binding to N. A complex of N and 1,5-IAEDANS-labeled RPS19 was titrated with unlabeled RPS19, and the fluorescence signal at 485 nm was recorded. The fluorescence intensity at 485 nm was plotted versus input concentration of the unlabeled RPS19. The inhibition constant, IC50, was calculated as described under “Experimental Procedures.” The experiment was repeated three times for the calculation of standard deviations.

### TABLE 1

| Buffer          | 80 mM NaCl | 160 mM NaCl | 320 mM NaCl |
|-----------------|------------|-------------|-------------|
| 50 mM Tris-HCl, pH 7.5 | 95.0 ± 3   | 97.0 ± 2    | 99.0 ± 1    |
| 50 mM HEPES, pH 7.5  | 92.0 ± 2   | 98.1 ± 1    | 103.4 ± 4   |
| 1× PBS, pH 7.5     | 97.0 ± 4   | 102.1 ± 1   | 99.2 ± 2    |

The fluorescence signal was 50% quenched with a 9-nm blue shift in the emission peak after covalent attachment to the cysteine side chain is due to shielding of the fluorescent moiety by RPS19. We observed a consistent decrease in the fluorescence quantum yield of 1,5-IAEDANS-labeled RPS19 with a 9-nm blue shift in the emission peak at saturating concentrations of N (Fig. 2A). The decrease in the fluorescence quantum yield and blue shift in the emission spectra are due to the interactions between N and RPS19. We did not notice any change in the fluorescence spectrum of 1,5-IAEDANS-labeled RPS19 at similar increasing concentrations of BSA, consistent with specific interaction between N and RPS19. A complex of N and 1,5-IAEDANS-labeled RPS19 resulted in the quenching of the 1,5-IAEDANS fluorescence signal (Fig. 2A). As expected, the complex formation between N and 1,5-IAEDANS-labeled RPS19 for binding to N. A complex of N and 1,5-IAEDANS-labeled RPS19 was titrated with unlabeled RPS19, and the fluorescence signal at 485 nm was recorded. As expected, the complex formation between N and 1,5-IAEDANS-labeled RPS19 for binding to N. A complex of N and 1,5-IAEDANS-labeled RPS19 was titrated with unlabeled RPS19, and the fluorescence signal at 485 nm was recorded.
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**FIGURE 3.** A, pH dependence of the N-RPS19 interaction. The binding reactions were carried out in binding buffer at different pH values. The $K_d$ values at each pH were calculated and plotted. Each $K_d$ value is a mean of three independent experiments. B, the data from Fig. 2C were replotted to determine the binding stoichiometry as described under “Experimental Procedures.” This analysis shows the binding of N and RPS19 in a 1:1 molar ratio. The results from fluorescence binding experiments performed under different conditions (Table 1) were similarly replotted (not shown), which revealed a similar binding stoichiometry. C, the results from A were verified by using a continuous variation plot (see “Experimental Procedures” for details), which confirmed the 1:1 binding between N and RPS19.

**Binding Stoichiometry and Energetics**—The fluorescence binding data from Fig. 2C were replotted for the calculation of binding stoichiometry as described under “Experimental Procedures.” This analysis showed that N and RSP19 bind with a molar ratio of 1:1 (Fig. 3B). We verified the binding stoichiometry using a continuous variation plot (Fig. 3C). The results from this analysis are consistent with the binding of a single molecule of N per molecule of RPS19. The thermodynamic parameters $\Delta G$, $\Delta H$, and $\Delta S$ were calculated using the van’t Hoff equation as described under “Experimental Procedures.” The binding experiments were performed at four different temperatures, and corresponding $K_d$ values were used to generate the van’t Hoff plot (Fig. 4). This analysis indicated that $\Delta G = -39.6 \pm 2.4$ kcal/mol, $\Delta H = -65.6 \pm 3.2$ kcal/mol, and $\Delta S = -87.2 \pm 2.7$ entropy units. From these thermodynamic data, it is evident that the binding between N and RPS19 is an enthalpy-driven process. The negative entropy change is compensated with a negative enthalpy change that drives the binding reaction with a total free energy change of $-39.6 \pm 2.4$ kcal/mol at 25°C.

**Binding of N Induces a Conformational Change in RPS19**—To further characterize the interaction between N and RPS19, we monitored the conformational change in RPS19, if any, due to the binding with N. We measured the accessibility of the 1,5-IAEDANS fluorophore before and after binding to N for acrylamide, a neutral molecule that quenches the 1,5-IAEDANS fluorescence signal. A fixed concentration of 1,5-IAEDANS-labeled RPS19 was incubated with increasing concentrations of acrylamide, and the quenched fluorescence intensity at 485 nm was recorded to generate the Stern-Volmer quenching plot (Fig. 5) as described under “Experimental Procedures.” This analysis revealed that the 1,5-IAEDANS fluorescent moiety at the C terminus of RPS19 is accessible to the neutral quencher with a $K_{sv}$ value of 4.3 $\pm$ 0.2. However, due to N binding, the $K_{sv}$ value was decreased to 2.5 $\pm$ 0.1 (Fig. 5), consistent with the 50% reduction in the accessibility of the 1,5-IAEDANS fluorescent moiety for acrylamide after the N-RPS19 interaction. This observation is consistent with the blue shift in the fluorescence emission peak of 1,5-IAEDANS-labeled RPS19 after binding to N, suggesting a hydrophobic microenvironment for the 1,5-IAEDANS fluorescent moiety after the N-RPS19 interaction. It is likely that binding of N induces a conformational change in RPS19 that sequesters away the 1,5-IAEDANS fluorescent moiety from the protein surface. However, it is equally possible that the binding site for N is located at the C terminus of RPS19; as a result, the 1,5-IAEDANS fluorescent moiety is shielded after the N-RPS19 interaction.

**N-RPS19 Interaction Is Required for N-mediated Translation Initiation**—We have previously reported that N facilitates the translation of capped mRNAs. N binds to the mRNA cap and RPS19, a component of the 40 S ribosomal subunit. N efficiently...
loads the 40 S ribosomal subunits on capped mRNAs and facilitates their translation. However, it is obvious to question how the N-mediated translation initiation mechanism helps the translation of capped viral mRNAs. Hantaviral mRNAs are synthesized by viral RdRp from the negative sense viral RNA genome. All three mRNA transcripts contain a 5′ cap, and a trimeric N molecule binds to the 5′ terminus of the viral mRNA transcript, increasing the length of the 5′ UTR up to ~72 nucleotides. Using competitive translations assays, we have previously reported that N facilitates the translation of any mRNA that harbors the 5′ viral mRNA UTR. Further studies revealed that a monomeric N molecule binds to the 5′ cap, and a trimeric N molecule binds to the highly conserved triplet repeat sequence of the viral mRNA 5′ UTR. Binding of N at these two positions is required for the preferential translation of hantaviral mRNAs by the N-mediated translation initiation mechanism. However, the efficient loading of 40 S ribosomal subunits on mRNA molecules by N is central to the N-mediated translation initiation mechanism. Because N interacts with the 40 S ribosomal subunit via RPS19, it is likely that the N-RPS19 interaction mediates the efficient loading of 40 S ribosomal subunits on capped viral mRNAs during N-mediated translation initiation.

We synthesized SNV S segment mRNA encoding nucleocapsid protein by a T7 transcription reaction. The mRNA generated by T7 RNA polymerase contained appropriate 5′ and 3′ UTRs and matched the mRNA generated by viral RdRp. This mRNA molecule was translated in rabbit reticulocyte lysates in the absence or presence of bacterially expressed and purified N. As shown in Fig. 6 (lanes 1 and 2), this mRNA is poorly translated in the absence of exogenously added N. The addition of N to the rabbit reticulocyte lysates enhanced the translation of mRNA by 10-fold. To determine whether the N-RPS19 interaction has a role in the N-mediated translation initiation mechanism, we added increasing input concentrations of bacterially expressed and purified N. We expected that exogenously added RPS19 would engage N and competitively inhibit the interaction between N and RPS19 in the 40 S subunit, monitored by a decrease in N-mediated translation of viral mRNA. As shown in Fig. 6 (lanes 3–7), the increasing input concentrations of RPS19 inhibited the preferential translation of viral transcript by the N-mediated translation mechanism. This observation is consistent with the competition experiments performed in Fig. 2D. To rule out the interference of exogenously added RPS19 in the function of translation machinery, we added similar increasing concentrations of RPS19 to the translation mixture lacking N. As shown in Fig. 6 (lanes 8–13), we did not observe a noticeable change in the translation of viral mRNA in the presence or absence of RPS19. This clearly suggests that the N-RPS19 interaction is required for preferential translation of viral mRNAs by the N-mediated translation mechanism.

To determine whether the N-RPS19 interaction is required for preferential translation of hantaviral mRNAs in vivo, we...
cloned firefly luciferase gene with flanking 5' and 3' UTRs of SNV S segment mRNA in a T7 expression vector (Fig. 6C). The 3' terminus of viral mRNA UTR was defined by a hepatitis delta virus ribosome sequence that was appropriately cloned downstream of the 3' UTR as described under "Experimental Procedures." The BSRT7-5 cells, which stably express T7 RNA polymerase, were transfected with this construct, and luciferase expression was monitored using a luciferase assay. As shown in Fig. 6D, we observed a weak luciferase expression from this construct. However, when cells were cotransfected with plasmids expressing both luciferase and N, a 7-fold increase in luciferase expression was observed as expected (Fig. 6C). To delineate whether the N-RPS19 interaction facilitated the translation of luciferase mRNA generated from PLUC UTR plasmid, we cotransfected cells with a fixed concentration of luciferase and N expression plasmids but gradually increased the concentration of RPS19 expression plasmid and monitored the luciferase signal. The gradual increase in the concentration of RPS19 expression plasmid during transfection resulted in the corresponding increase of RPS19 protein in cells (not shown).

As shown in Fig. 6D, the gradual increase in RPS19 expression gradually reduced the luciferase signal in BSRT7-5 cells, suggesting that overexpressed RPS19 likely competed with RPS19 recruited in the 40 S subunit for binding to N. This competitive inhibition would decrease the number of N-associated 40 S subunits and inhibit the N-mediated translation initiation mechanism as evident from a gradual decline in luciferase signal (Fig. 6C). To rule out the possibility that overexpression of RPS19 specifically interferes with the N-mediated translation initiation mechanism and does not perturb the cellular translation apparatus, we repeated the above experiment without the inclusion of N expression plasmid in cotransfection. We did not observe a noticeable change in the luciferase expression with the overexpression of RPS19 in cells. These observations are consistent with the idea that the N-RPS19 interaction is required in the N-mediated translation initiation mechanism.

**DISCUSSION**

Viruses are under pressure to maintain a small genome size for rapid multiplication in host cells and thus do not carry genetic information to encode the components of translation machinery, which requires huge genome space. Viruses are dependent upon the host cell translation machinery for the translation of their mRNAs. To avoid the competition of host cell mRNAs for the same translation apparatus, viruses have adopted selfish strategies that selectively favor the translation of viral mRNAs. For example, one such well-characterized mechanism is the internal ribosome entry site, a cis-acting element in the viral mRNA that selectively loads ribosomes on viral transcripts. We have recently found that hantaviruses initiate the translation of their capped mRNAs by a trans-acting mechanism operated by viral N. N binds to both the mRNA 5' cap and the 40 S ribosomal subunit via RPS19. The N-RPS19 interaction likely facilitates the ribosome loading on capped mRNAs during translation initiation. Although the N-mediated translation initiation mechanism in general facilitates the translation of capped mRNAs, we have recently reported that N specifically binds to a highly conserved triplet repeat sequence of the viral mRNA 5' UTR and preferentially loads ribosomes on viral transcripts to favor their translation in host cells where cellular transcripts are competing for the same translation apparatus. The central aspect of N-mediated translation initiation is the interaction between N and RPS19 in the 40 S ribosomal subunit followed by efficient loading of 40 S subunit onto the mRNA.

Like other ribosomal proteins, RPS19 is synthesized in the cytoplasm and translocated to the nucleus where it participates in ribosome biogenesis. The RPS19 is mainly found associated with the 40 S ribosomal subunit. It might be possible to incorporate 1,5-IAEDANS-labeled RPS19 into the ribosome and assess ribosome function. To examine the role of the N-RPS19 interaction in N-mediated translation initiation, we used a fluorescence spectroscopic assay to quantitatively estimate the binding affinity between N and RPS19. This sensitive assay yielded a $K_d$ value of $95 \pm 3$ nM for the N-RPS19 interaction, although a different $K_d$ for the interaction between N and RPS19, which is recruited in the 40 S subunit, cannot be ruled out. It is equally possible that N undergoes post-translational modification in eukaryotic cells, and modified N might have different affinity for RPS19. Although these studies demonstrate the significance of the N-RPS19 interaction in N-mediated translation initiation, the mechanism for ribosome loading in this translation strategy is still a mystery. We have previously reported that a single molecule of N interacts with the mRNA 5' cap. In addition, N forms homodimers and homotrimers in cells (8). It is possible that dimerization of N molecules individually bound to the mRNA cap and RPS19 mediates the loading of 40 S subunit on capped mRNA during N-mediated translation initiation (Fig. 7), although the studies reported here do not provide any evidence for such dimerization. On the basis of the crystal structure of RPS19 from *Pyrococcus abyssi*, cryoelectron microscopy (cryo-EM) mapping, and the x-ray structure of the yeast ribosome, the location of RPS19 has been mapped to the head region of the 40 S subunit where it is accessible for interaction with other molecules (61, 62). It is likely that the N-RPS19 interaction would recruit N at the head region of the 40 S subunit. Once the initiating AUG codon is recognized on the mRNA during translation initiation, the 60 S subunit then
joins the head region of the 40 S subunit, and a translation-competent 80 S ribosome is formed. Further studies are required to determine whether N dissociates from the 40 S subunit during translation initiation or elongation.

N is a capsid protein with a basic function to package viral genomic RNA. In vitro studies have shown that N binds to the viral genomic RNA with a $K_d$ value of 25 nm, and we also previously reported that N binds to the mRNA cap with a $K_d$ value of 120 nm (50). N is the first protein expressed in cells and becomes saturated shortly after viral infection. It is likely that after expression N interacts with different targets in the cell cytoplasm with a priority based on affinity. Because the binding affinity of N with RPS19 is comparable with viral genomic RNA and the mRNA 5' cap, it is possible that the N-RPS19 interaction reserves a small population of host ribosomes that remains dedicated for the selective translation of viral mRNAs in cells. Unlike other viruses, such as flu or encephalomyocarditis virus, hantaviruses do not cause host translation shutoff and cytopathic effects to the host cell. Hantaviruses replicate efficiently in host cells, suggesting that their mRNAs are translated efficiently in the cell cytoplasm where cellular transcripts are competing for the same translation machinery. However, translation of viral S segment mRNA in rabbit reticulocyte lysates showed weak expression in the absence of exogenously added N (Fig. 6C). N expression from mRNA in rabbit reticulocyte lysates is negligible and should not be confused with the comparatively higher concentration of exogenously added N in the translation reaction. This observation suggests that hantaviral mRNAs rely on the N-mediated translation initiation mechanism that lures the host cell translation apparatus for their preferential translation in cells.

RPS19 was initially considered to have only a structural role in ribosome biogenesis until its loss-of-function mutations were identified in patients with a rare hematological disease, Diamond-Blackfan anemia (63). Recently RPS19 has been found to interact with cellular protein from diverse protein families, including NTPases, hydrolases, isomerases, kinases, splicing factors, transcription factors, transferases, and DNA/RNA-binding proteins (64). Although the biological significance of such diverse interactions of RPS19 is still unknown, it is likely that the N-RPS19 interaction will have an effect upon other functions of RPS19. Further studies, including the effect of down-regulation of RPS19 upon hantavirus replication, are required to understand the role of the N-RPS19 interaction in the hantavirus life cycle.

We have previously reported that N initiates the mRNA translation without the requirement of the eIF4F complex, an amalgam of three initiation factors: eIF4A, eIF4E, and eIF4G (17). It has been shown that translation of influenza virus mRNA does not require eIF4A, a critical component of the eIF4F complex (65). It is possible that minus-stranded RNA viruses with a segmented genome have evolved similar mechanisms to facilitate the translation of their mRNAs in host cells. Our studies demonstrate that the N-RPS19 interaction plays a critical role in the N-mediated translation initiation mechanism. We have developed a very sensitive fluorescence assay to study the interaction between N and RPS19. This assay can be further developed to screen chemical libraries in high-throughput mode for the identification of molecules that inhibit the N-RPS19 interaction. Such chemical inhibitors might block the replication of a broad spectrum of segmented negative-stranded RNA viruses.

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