

Binding to selected regions of reovirus mRNAs by a nonstructural reovirus protein

(3' terminus/mRNA selection/filter binding/protected fragment)

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ABSTRACT When assembled into 13-19S particles, the reovirus nonstructural protein σ -NS selectively binds single-stranded RNAs. Sedimentation analyses combined with binding to nitrocellulose membrane filters showed that 1-2 pmol of reovirus mRNAs from the large, medium, or small size classes saturated *in vitro* the binding site(s) on 13-19S particles containing 100 pmol of σ -NS. All mRNA segments in each size class bound to particles, and no mRNAs in one size class excluded the binding of mRNAs in any other class. In competition experiments, the maximal binding of all reovirus mRNAs to particles of σ -NS was achieved when medium and small mRNAs were bound before the large mRNAs. This preferred order of addition of mRNAs to σ -NS resulted in a marked increase in the size of some of the complexes. This finding suggests that the addition of large mRNAs last to particles promoted the formation of complexes with more than one RNA segment bound per particle. The 13-19S particles of σ -NS protected 20- to 40-nucleotide RNA fragments from nuclease digestion. At least one of the protected fragments from mRNAs of each size class included the 3' terminus; the remaining were from internal regions of the mRNAs. The protected RNA fragments rebound to particles during a second or third cycle of binding in a configuration in which they were fully protected from nuclease digestion. We conclude that binding of particles of σ -NS to reovirus mRNAs was not at random sites but was to specific regions unique for members of each size class.

Reovirus single-stranded (ss) RNAs of the same polarity as mRNAs are templates for synthesis of minus strands of double-stranded (ds) genome RNA (1). To produce an infectious virus particle, 10 different ss RNA segments must be selected and condensed in a particle in which replication occurs. Three proteins support reovirus ds RNA synthesis, as there are three separate groups of ds RNA negative mutants. Two, σ -2 and λ -3, are also structural components of the reovirus core. The third is the nonstructural (NS) protein, σ -NS (2-4).

In our continuing analysis of reovirus ds RNA synthesis, we reported that virus-specific particles, 11-12 nm in diameter, accumulated during infection at 30°C with the maximal amount present 10 hr before onset of exponential growth of virus (5). These small particles sedimented at 13-19 S, were composed solely of σ -NS, had poly(C)-dependent poly(G) polymerase activity, and bound different ss RNAs (5, 6). Binding of ss RNAs and polymerase activity were properties of σ -NS only when in 13-19S particles, as neither activity was retained in the 4-5S subunits of particles.

Competition experiments revealed differences in binding affinity for ss RNAs amongst the 13-19S particles. Some preferentially bound poly(C); others bound reovirus ss RNAs; and still others bound both templates with about equal affinity (6).

Among the RNAs which bound to particles of σ -NS, polyuridylylate and reovirus ss mRNAs were the most potent inhibitors of the poly(G) polymerase activity. Yet once bound, neither poly(U) nor reovirus mRNAs were copied *in vitro*.

What is the role of σ -NS in reovirus ds RNA synthesis? In extracts from infected cells, σ -NS was the only reovirus protein complexed to reovirus ss RNAs (7). Temperature-shift experiments revealed that σ -NS synthesized very early in infection was sufficient for the production of full yields of infectious reovirus. Crucial to an understanding of the function of σ -NS is the determination of the site on reovirus ss RNAs to which it binds. Do the particles recognize a particular sequence or secondary structure of the 10 individual ss RNAs? Demonstration of binding to the 3'-end of the ss RNAs would be obligatory if the particles were one component of the replicase, but binding to a selected region also would be expected for a principle used for recognition or condensation. We report that binding of particles of σ -NS to the reovirus ss RNAs was not random but was to specific regions unique for members of each size class.

EXPERIMENTAL PROCEDURES

Preparation and Purification of 13-19S Particles. These particles comprised only of σ -NS were purified from cytoplasmic extracts obtained from L929 cells infected with reovirus at 30°C for 22 hr (5). After sedimentation into a glycerol cushion, material from the cytoplasmic extract was centrifuged sequentially into CsCl and glycerol density gradients. The 13-19S particles, free of RNA and all proteins but σ -NS, were identified by their poly(C)-dependent poly(G) polymerase activity (5), pooled, and stored at -28°C. The portion of pooled particles standardly used contained 1.5 μ g (30 pmol) of σ -NS in 0.23 ml.

Preparation of Reovirus ss RNAs. Capped and methylated reovirus ss RNAs were synthesized *in vitro* by reovirus cores as described (8) but were modified by addition of S-adenosylmethionine to 0.02 mM. The mRNAs were labeled uniformly with 32 P or 3 H to a specific activity of $3-5 \times 10^5$ cpm/ μ g by using [32 P]CTP at 0.578 Ci/mmol and [32 P]UTP at 0.193 Ci/mmol or [3 H]CTP at 1.85 Ci/mmol and [3 H]UTP at 0.658 Ci/mmol (1 Ci = 3.7×10^{10} becquerels). Separately, mRNAs were labeled at their 5' end to a specific activity of $0.8-1.5 \times 10^4$ cpm/ μ g with S-adenosyl-L-[methyl- 3 H]-methionine (9). After separation into large (l; 24 S), medium (m; 19 S), and small (s; 14 S) size classes (8), the mRNAs in each class were pooled separately and recentrifuged through sucrose gradients before use. Unlabeled mRNAs were labeled with [$5'$ - 32 P]pCp at their 3' terminus (10) to a specific activity of $2-3 \times 10^5$ cpm/ μ g. After 16 hr at 4°C in the reaction mixture, the mRNAs were extracted with phenol, chromatographed through Sephadex G-50, and bound to and eluted from Whatman cellulose CF-11 (11).

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Abbreviations: ss, single stranded; ds, double stranded; NS, nonstructural; s, small; m, medium; l, large.

Competition Between Reovirus mRNAs for Binding to Particles of σ -NS. The standardly used portion of particles containing 1.5 μ g (30 pmol) of σ -NS and the saturating amount of the first-bound mRNA(s) were incubated in 0.5 ml of binding buffer (10 mM Tris-HCl, pH 8.1/0.02% Triton X-100/2 mM MnCl₂/7 mM mercaptoethanol). This amount of particles was saturated by 0.46 μ g of l-, 0.38 μ g of m-, or 0.21 μ g of s-mRNAs. After 10 min at 12°C, the saturating amount of the competing mRNA(s) was added for an additional 10 min, and the mixture was centrifuged through a 15–30% sucrose gradient in binding buffer in an SW 41 rotor at 40,000 rpm for 4.3 hr at 4°C. Equal fractions, 30, collected from below were analyzed for radioactivity or for complex bound to Schleicher and Schuell 23-mm BA85 membrane filters (6). The microgram amount of RNA bound to σ -NS was determined from the amount of radioactively labeled mRNA retained on filters divided by the specific activity of the particular mRNA. The picomole amount of RNA was calculated from the micrograms of bound mRNA divided by the average molecular weights of 1.3×10^6 , 0.7×10^6 , and 0.4×10^6 for l-, m-, and s-mRNAs, respectively.

σ -NS-Protected mRNA Fragments. Preparation. The l-, m-, or s-[³²P]mRNAs bound to the 13–19S particles of σ -NS were treated for 15 min at 12°C with RNase T1 at 100 μ g/ml, and the mixture was centrifuged through 15–30% sucrose gradients in binding buffer at 40,000 rpm for 15 hr at 4°C. The bound mRNA fragments were concentrated by ethanol precipitation and extracted with phenol. Where noted, mRNA fragments were subjected to a second and even a third cycle of binding, nuclease treatment, and sedimentation in sucrose gradients.

Analysis. After phenol extraction, the protected [³²P]mRNA fragments were separated by electrophoresis in slab gels of 20% acrylamide/0.67% bisacrylamide in 7 M urea/90 mM Tris borate, pH 8.3 (12). Electrophoresis in 90 mM Tris borate, pH 8.3/1 mM EDTA was carried out until the xylene cyanol marker had migrated 11 cm from the origin. An additional marker, kindly supplied by Stephen Plotch, was the fragment of 39 nucleotides generated by RNase T1 treatment of alfalfa mosaic virus RNA 4 containing ³²P in its 5'-terminal cap 1 structure (13).

Materials. Labeled precursors were from New England Nuclear and Amersham. The T4 RNA ligase was from P-L Biochemicals, and RNase T1 was from Calbiochem.

RESULTS

The amounts of the reovirus l-, m-, and s-mRNAs that saturated the binding site(s) of a given number of particles of σ -NS were used in competition experiments to identify (i) if the binding of particles to mRNAs of the three size classes was optimized by a particular order of addition and (ii) if complexes formed that contained more than one mRNA per particle. Less than saturating amounts were used to recover specific nuclease-resistant fragment(s) from each size class.

Capacity of Particles of σ -NS to Bind the Reovirus l-, m-, and s-mRNAs. When reovirus mRNAs of each size class (24, 19, and 14S) were incubated with the 13–19S particles of σ -NS at 12°C in the presence of 2 mM Mn²⁺, complexes of RNA and protein formed that were retained on filters (6). All mRNAs within a size class remained on filters in a complex with σ -NS until the saturating amount of mRNAs was exceeded (data not shown). Free mRNAs were not retained (6). The complexes formed with the l-, m-, or s-mRNAs sedimented faster in sucrose gradients than the respective RNAs or the 13–19S particles did alone (Fig. 1 A and B). As complexes with mRNAs from each size class were distinguishable from each other in gradients and from free mRNAs in the filter binding assay, the

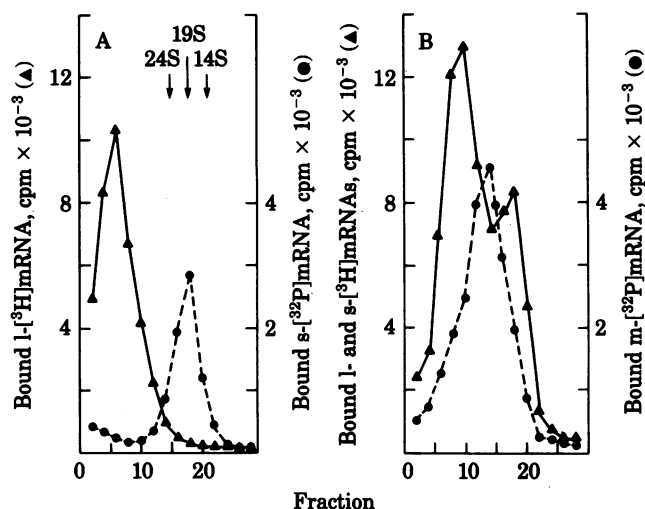


FIG. 1. Comparison of size in 15–30% sucrose gradients of complexes formed by 13–19S particles of σ -NS and reovirus mRNAs from each of the three size classes. Particles of σ -NS were incubated with saturating amounts of s-[³²P]mRNA and l-[³H]mRNA (A) or m-[³²P]mRNA and l- and s-[³H]mRNA (B). The amount of labeled mRNA complexed to particles of σ -NS was determined by filter binding and is shown on the ordinate. Binding conditions, sedimentation analysis, and filter binding are described. The direction of sedimentation was from right to left.

amount of l-, m-, or s-mRNAs bound to σ -NS could be determined by using (l + s)-[³H]mRNAs and m-[³²P]mRNAs. In this approach, complexes of l-mRNAs with particles must separate from those with s-mRNAs with little overlap, and they did (Fig. 1A). Complexes with l-[³H]mRNAs sedimented markedly faster than those with s-[³²P]mRNAs; and complexes with either clearly separated from those with particles that had bound m-mRNAs (Fig. 1B). By summing the radioactivity in each complex obtained after filter membrane binding, we found that 1.2, 1.8, and 1.7 pmol (or 1.5, 1.3, and 0.7 μ g) respectively of l-, m-, and s-mRNAs saturated *in vitro* the binding site(s) on 13–19S particles composed of 100 pmol (5 μ g) of σ -NS (Table 1).

If σ -NS in the 4–5S subunits and in 13–19S particles has similar shape and density, respectively, as the 4.8S and 16S oligomeric protomers of picornaviruses, the approximate molecular weights of subunits and particles of σ -NS will be 95,000 and 570,000 (14). As the molecular weight of σ -NS is 50,000 (8), the 4–5S subunits would be dimers, and the 13–19S particles would contain six dimers. Thus, 100 pmol of σ -NS (Table 1) would

Table 1. Reovirus mRNAs bound to σ -NS in 13–19S particles

Conditions		mRNAs bound,*			
First-bound mRNA(s)	Competing mRNA†	(pmol RNA/100 pmol σ -NS)			Total
		l	m	s	
l	None	1.2	—	—	1.2
m	None	—	1.8	—	1.8
s	None	—	—	1.7	1.7
l	m	0.9	0.9	—	1.8
l	s	1.0	—	0.7	1.7
l + m + s	None	0.8	0.6	0.4	1.8
l + m	s	0.9	0.8	0.6	2.3
l + s	m	1.0	0.5	0.7	2.2
m + s	l	1.1	1.1	0.9	3.1

* The maximal amount of each bound mRNA labeled with ³H or ³²P is shown.

† Order of addition: (i) first-bound mRNA(s); (ii) binding buffer; and (iii) competing mRNA.

contain 30×10^{12} dimers in 5×10^{12} particles. To saturate this number of particles, 0.7, 1.1, and 1.0×10^{12} molecules of l-, m-, and s-mRNAs, respectively, were needed. When mRNAs of one size class were present, the stoichiometry of the binding reaction suggested that, at saturation, there were five particles present for each mRNA.

When mRNAs of two size classes were incubated with particles and analyzed as in Fig. 1, some of each bound, but the total amount did not exceed the picomoles bound at saturation when each was added singly (Table 1; Fig. 1). As only saturating amounts of first-bound and competing RNAs were used, the difference in the amount bound from the saturating level reflects the amount of first-bound mRNA displaced and the amount of competing mRNA prevented from binding. When mRNAs in the three size classes were added together followed by Mn^{2+} , a condition giving each an equal opportunity to bind, some of each did bind but at levels 24% of maximum for the s-mRNAs and 67% of maximum for the l-mRNAs. There was a preferential tendency for l-mRNAs to be bound over m-mRNAs over s-mRNAs (Table 1). Similarly, the total amount bound did not exceed the saturating value of 1.8 pmol of m-mRNAs bound. However, this value was exceeded by 1.3 pmol when the mRNAs were added sequentially in a definite order—namely s- and m-mRNAs bound before binding of l-mRNAs. This increased value of 3.1 pmol bound suggests either that particles that had not previously bound mRNAs now did or, alternatively, that individual particles had bound more than one mRNA. The latter appears to be the case. When m- and s-mRNAs were first bound to particles of σ -NS followed by l-mRNAs, we found that complexes containing the m- and s-mRNAs had increased in size and sedimented substantially faster than the complex formed with the m- or s-mRNAs alone (data not shown). This finding suggests that particles had bound more than one mRNA, but we could not distinguish if the additional mRNA(s) bound to particles was from the same or a different size class.

Characterization of Fragments from the l-, m-, and s-mRNAs Protected from Nuclease Digestion. Do uniformly labeled mRNAs of each size class, when bound to particles, yield RNA fragments protected from digestion with RNase T1? As there are 3, 3, and 4 unique mRNAs in the l, m, and s size classes (8), we could expect at least 3, 3, and 4 protected fragments, respectively, from each if binding was at specific regions. When less than saturating amounts of uniformly labeled l-, m-, and s-mRNAs were bound separately to 13–19S particles and treated with RNase T1, 10–15% of the radioactivity in the originally committed mRNAs was in RNase T1-resistant fragments, which cosedimented at 13–19S with particles (Fig. 2). Only half of the fragments sedimenting with particles of σ -NS were specifically bound, as the labeled mRNA fragments retained with particles on nitrocellulose filters contained 5–9% of the input ^{32}P -labeled material. No ^{32}P -labeled fragments in fractions 20–29 (Fig. 2) were retained on filters, even if particles of σ -NS were added before filtration.

To confirm that only some mRNA fragments in fractions 1–19 (Fig. 2) were bound specifically and to identify the basis for the difference in sedimentation value of material in the pellet and gradient fractions 1–10 from that in fractions 11–19, we separated the complexes into fractions 1 and 2 (shown for complexes with m-mRNAs, Fig. 2B). The RNA fragments in the pooled fractions 1 and 2 were freed of particles of σ -NS by extraction with phenol and separately subjected to a second binding cycle. After binding, half of each was treated with RNase T1. RNA fragments rebinding and cosedimenting with particles contained about 5% of the input ^{32}P -labeled material when not treated with RNase T1 (Fig. 3 A and C); when treated with RNase T1, the number was reduced to 1–2% (Fig. 3 B and D).

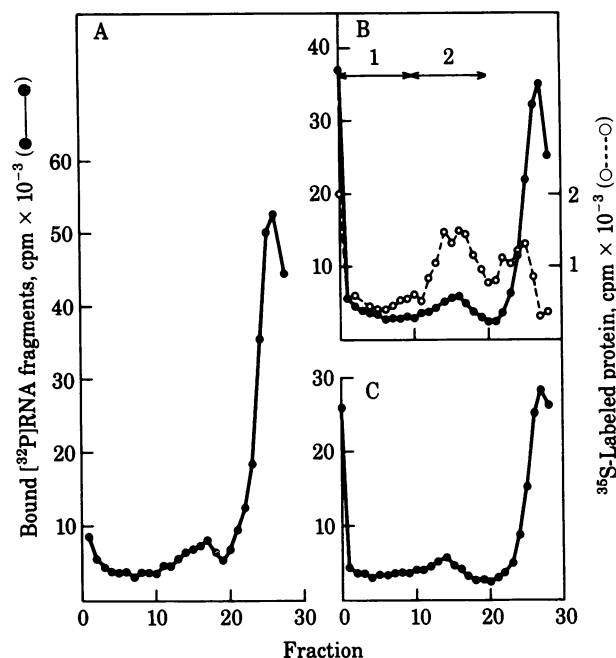


FIG. 2. Sedimentation in 15–30% sucrose gradients of nuclelease-resistant mRNA fragments bound to 13–19S particles. Less than a saturating amount of mRNA in l-mRNA (A), m-mRNA (B), and s-mRNA (C) was bound to particles of σ -NS and treated with RNase T1 at 100 μ g/ml. After centrifugation at 40,000 rpm for 15 hr at 4°C, 30 equal fractions were collected from below. A portion of each was taken to determine total radioactivity in ^{32}P RNA fragments. Material in each gradient was separated into fractions 1 and 2, as indicated in the top right panel. The distribution of ^{35}S -labeled σ -NS is shown only for complexes with RNA fragments from m-mRNAs (B) but was similar for complexes with RNA fragments from l- and s-mRNAs.

The data are shown only for m-mRNA fragments, but similar results were obtained with l- and s-mRNA fragments. After the second cycle, bound RNA fragments from fractions 1 and 2 both sedimented with particles from 13 to 19 S, and few complexes sedimented faster than 19 S. The basis of the initial difference in sedimentation of material in fractions 1 and 2 is not known, but the difference may reflect protected fragments of larger size in fraction 1 after the first RNase T1 treatment.

When analyzed on membrane filters after the second binding cycle, greater than 90% of RNA fragments from fractions 1 or 2 sedimenting with particles of σ -NS were retained on filters (data not shown). That these bound RNA fragments cosedimenting with particles were specific was reconfirmed by identification that all fragments rebound during a third binding cycle and were fully protected from nuclease digestion.

Analysis on gels of RNA fragments from fraction 1 or 2, which had been bound and nuclease-treated two or three times, yielded reproducibly 5, 4, and 5 major oligonucleotides from the l-, m-, and s-mRNAs, respectively (Fig. 4, uniformly labeled, lanes b). Protected RNA fragments were 24–38 nucleotides long. After only one binding cycle, protected fragments were longer, but included were fragments identical in length to those rebound after the second or third cycle.

3' Terminus of mRNAs Among Protected Fragments. To determine if any protected fragments contained either end of intact mRNAs, the 5' end of mRNAs was labeled during *in vitro* synthesis with S-adenosyl-L-[methyl- 3H]methionine. The 3' terminus of mRNAs in a different preparation was labeled with [^{32}P]pCp after their synthesis and separation into three size classes. After binding and nuclease treatment, fragments containing 15–30% of the initial 3'-end ^{32}P label remained on filters

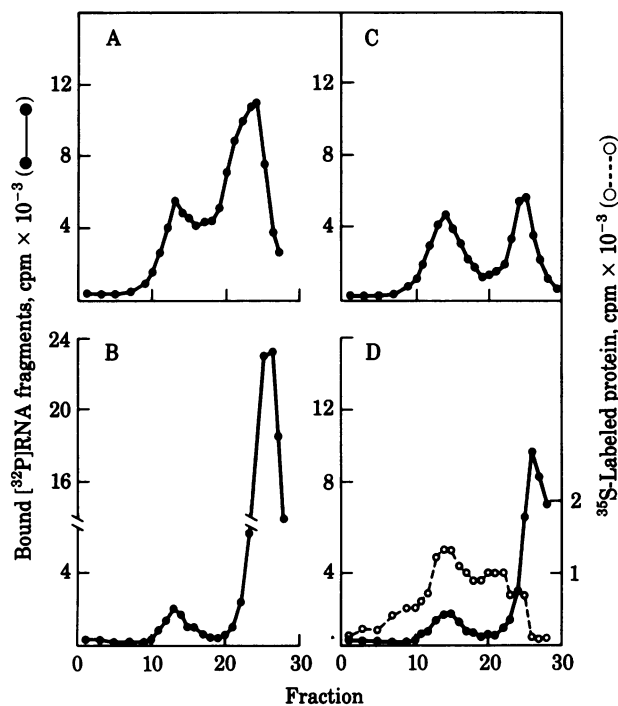


FIG. 3. Rebinding to 13-19S particles by protected RNA fragments from fractions 1 and 2 of Fig. 2. (A and B) Fraction 1 fragments, untreated (A) or treated (B) with RNase T1. (C and D) Fraction 2 fragments, untreated (C) or treated (D) with RNase T1. The fragments used in binding were those from m-mRNAs of Fig. 2, but identical results were obtained when fragments from l- or s-mRNAs were used. RNA fragments in fractions 1 and 2 of Fig. 2 were concentrated by ethanol precipitation, extracted with phenol, and rebound to particles of σ -NS. After binding, the fragments were either untreated (A and C) or treated with RNase T1 (B and D) and centrifuged through sucrose gradients as in Fig. 2.

with particles (Table 2). A minimal amount of [3 H]methyl-labeled 5'-capped fragment was reproducibly bound after one cycle, but this did not rebound during a second cycle.

Analysis of the 3'-end-labeled fragments on gels revealed that the uniformly labeled l-mRNA 27- and 35-nucleotide fragments

Table 2. Capacity of σ -NS to bind and protect either end of reovirus mRNAs from RNase T1 digestion*

mRNAs	3'-end-labeled [32 P]mRNAs, cpm $\times 10^{-2}$		5'-end-labeled [methyl- 3 H]mRNAs, cpm $\times 10^{-2}$	
	Total bound	Protected	Total bound	Protected
l	5,990	1,730 (29)	892	19 (2)
m	8,740	1,980 (23)	990	14 (1)
s	15,400	2,100 (14)	948	27 (3)

* Conditions for binding mRNAs to particles of σ -NS, for subsequent treatment with RNase T1 and for analysis by filter binding after centrifugation, were those described in Fig. 2; 15 μ g of particles of σ -NS was used for binding separately 7.2, 5.6, and 4.2 μ g, respectively, of 5'-end-labeled l-, m-, and s-[methyl- 3 H]mRNAs and for binding separately 5.4 μ g each of mRNAs labeled at their 3'-end with [32 P]pCp. Radioactivity in nuclease-resistant mRNA fragments is shown in parentheses as the percentage of mRNA initially bound.

included the 3' terminus of l-mRNAs; the 24-, 29-, and 38-nucleotide fragments were from an internal region of l-mRNAs (Fig. 4). The 35-nucleotide m-mRNA fragment included the 3' terminus; the 24-, 29- and 38-nucleotide m-mRNA fragments were from internal regions. The 25-, 36- and 38-nucleotide s-mRNA fragments included the 3' terminus. The 24- and 35-nucleotide mRNA fragments were from internal regions of s-mRNAs.

The pattern of the s-mRNA fragments differed from those of l- and m-mRNAs in relative amounts and in sizes of the 3'-end-labeled fragments. Although, the protected RNA fragments from l- and m-mRNAs were similar in size, uniqueness was supported by finding that the patterns of protected fragments obtained after one binding cycle were different; after two binding cycles, the 29-nucleotide fragment was the most prominent band from l-mRNAs and the least prominent from m-mRNAs. Moreover, after one cycle of binding of the 3'-end-labeled mRNAs, the 27-nucleotide fragment was the most prominent from l-mRNAs, whereas the 35-nucleotide fragment was the most prominent from the m-mRNAs.

As reovirus mRNAs band diffusely in polyacrylamide gels, their fractionation has been difficult to achieve. Sequence anal-

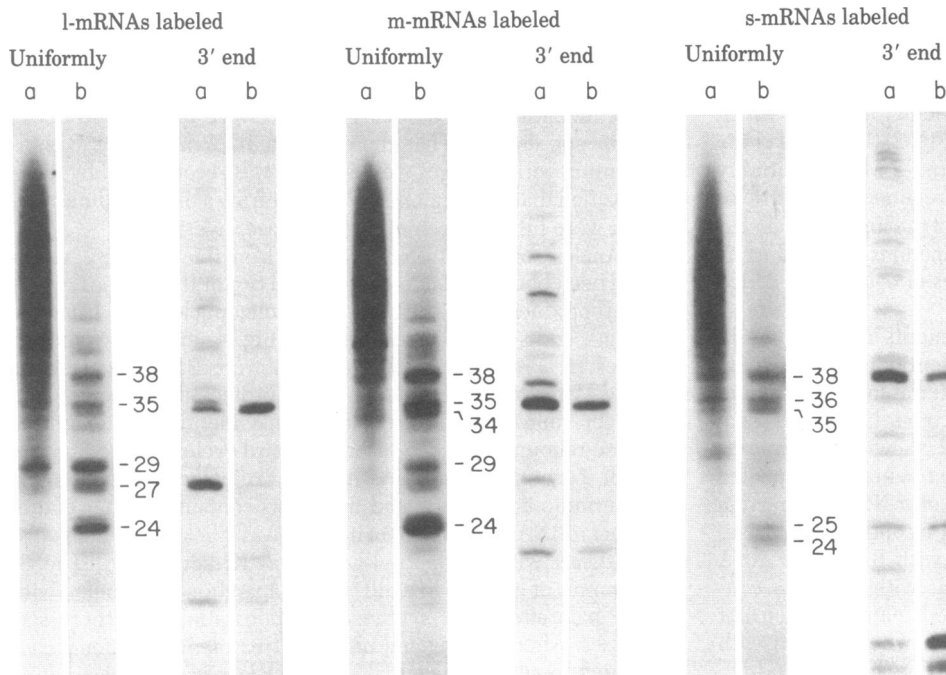


FIG. 4. Size in 20% polyacrylamide slab gels of fragments from uniformly 32 P-labeled or 3'-end-labeled l-, m-, and s-mRNAs bound to particles of σ -NS and protected from digestion by RNase T1. Lanes: a, fragments obtained after one cycle of binding and treatment with RNase T1; b, fragments after two cycles of binding and treatment with RNase T1. After a third cycle of binding and treatment with RNase T1, the gel pattern obtained was similar to that in lanes b. Markers during electrophoresis were xylene cyanol (for 28 nucleotides in this system) and 32 P-capped fragment after treatment of alfalfa mosaic virus RNA with RNase T1 (39 nucleotides). For clarity in comparison to uniformly labeled bands, we have adjusted the 3'-end-labeled lanes to reflect the nucleotide lengths of the native fragments minus the pCp which had been added to the 3'-end.

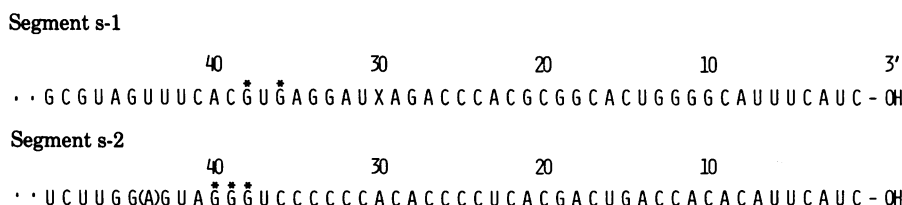


FIG. 5. The 3'-terminal sequences of the s-1 and s-2 RNA plus strands of reovirus type 3 (16, 17). The asterisks (*) indicate possible cleavage site(s) to yield the 3'-end fragment protected by σ -NS from RNase T1 digestion.

ysis of the 3'-end of the genome plus strands has not yielded unequivocal data for all 10 mRNAs, perhaps due to secondary structure at this end. The 3'-end sequence of segments l-3, m-3, s-1, s-2, s-3, and s-4 is known for 11 or 12 nucleotides (15). Only for s-1 and s-2 does the known sequence extend beyond (Fig. 5, obtained from data in refs. 16 and 17). If the 3' end of segments s-1 and s-2 yields a protected fragment, the asterisks in Fig. 5 denote the location(s) of cleavage by RNase T1 to yield the 36- or 38-nucleotide s-mRNA fragments. It would be expected that neither s-1 or s-2 yielded the 25-nucleotide fragment as there is no guanosine residue at this location.

As some fragments from mRNAs in each size class came from internal regions of the mRNAs, it remains to be determined if the 13-19S particles of σ -NS bind only to one region on an individual mRNA and to more than one region in a second mRNA in the same size class, e.g., a two-point attachment to the 3' end and to a region located internally on the same mRNA.

DISCUSSION

The earliest intermediates in the assembly of reovirus replicase particles have eluded identification. Replicase particles thus far identified had most RNA already fully double stranded and had acquired most proteins of complete virus. Prime needs are to identify early particles in which most RNA is single-stranded and to identify the earliest complex that selects and condenses 10 different reovirus ss RNAs for replication. Selection is efficient, as there are reovirus preparations in which the ratio of particle to plaque-forming unit approaches unity. It is possible that the binding capacity for nucleic acids of the 13-19S particles of σ -NS described here plays a key role in condensation of the ss RNAs. Binding at the 3' end of mRNAs is consistent with particles of σ -NS being the condensing principle or a component of it but is also consistent with particles being a component of the reovirus replicase. The binding at one time of more than one mRNA per particle supports the role of particles in condensation of reovirus mRNAs.

In cellular extracts, σ -NS was the only viral protein found complexed to all reovirus mRNAs at all early times of infection and after use of different methods to disrupt cells (7). The complexes were not isolated in pure form, but their molar ratio of σ -NS to reovirus mRNAs was estimated to be at least 20. If the complexes in extracts had σ -NS in particles each with six dimers, there would be about 1.7 particles per mRNA to compare with our molar ratio of 5 when one size class of mRNAs was used *in vitro* and 2.6 when binding of l-mRNAs followed that of m- and s-mRNAs. As the efficiency of the *in vitro* binding reaction

approaches that found for complexes from infected cells, the basis for the excess of particles to mRNAs remains to be determined. It must be emphasized that the number of reovirus mRNAs that can bind to one particle and the least number of particles required to bind 10 different RNA segments are not known. Our results suggest that, dependent on the order of addition of mRNAs, more mRNAs are bound to particles and more than one mRNA can be bound to one particle.

Particles of σ -NS protected from nuclease digestion the 3' terminus of mRNAs in each size class in addition to fragments unique in size from an internal region. We cannot be certain that when binding conditions have been optimized, the same or a larger percentage of the 3' terminus of added mRNAs will be protected. Optimization of binding may require that the 10 reovirus mRNAs be added in a particular order rather than as members of one size class as done in our experiments. Our report points to the 3' end of mRNAs as one site recognized by particles of σ -NS. It is premature to suggest the basis for this recognition until longer sequences at this end of more of the mRNAs become known.

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