Synthesis in *Escherichia coli* of the Reovirus Nonstructural Protein σNS

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The coding region of reovirus type 3 genomic segment S3, encoding the nonstructural protein σNS, was placed under the control of the bacteriophage λ p1 promoter in the *Escherichia coli* expression plasmid pRC23 (J. C. Lacal, E. Santos, V. Notario, M. Barbacid, S. Yamazaki, H.-F. Kung, C. Seamans, S. McAndrew, and R. Crowl, Proc. Natl. Acad. Sci. USA 81:5305–5309). Derepression of the p1 promoter led to the synthesis of a protein of the same molecular weight as σNS produced in reovirus-infected L cells. The expressed protein was indistinguishable from authentic σNS by peptide mapping with *Staphylococcus aureus* V8 protease and by immunoblot analysis. Most importantly, the purified protein had nucleic acid-binding properties similar to that previously shown for σNS obtained from infected cells. Binding of single-stranded RNAs by recombinant σNS protein was inhibited by GTP.

The reovirus genome consists of 10 segments of double-stranded RNA (dsRNA) (24). The segments are transcribed by virus-associated RNA polymerase to form capped mRNAs (4) which also function as templates for a putative replicase in virus-infected cells (14, 28). Each dsRNA segment codes for at least one protein. Two of these, rNS and σNS, encoded by genomic segments M3 and S3, respectively, are found only in infected cells (11, 30). While the function of these two nonstructural proteins is unknown, there is some evidence to suggest that σNS may act in the selection and condensation of the 10 different single-stranded RNAs (ssRNAs) into precursor subviral particles in preparation for dsRNA synthesis (7, 11, 29). Thus, Huismans and Joklik (11) found that σNS has the unique capacity among reovirus-encoded proteins to bind ssRNAs. It has since been shown that virus-specific particles sedimenting at 13S to 19S were composed solely of σNS (7, 8) and protected 20- to 40-nucleotide RNA fragments of reovirus mRNAs, including 3′ termini, from nuclease digestion (6). In addition, it is known that σNS plays an important role early in the reovirus replication cycle, as a temperature-sensitive mutant defective in σNS protein was unable to synthesize dsRNA at the restrictive temperature (13, 20, 23).

It has also been demonstrated that σNS in 13S to 19S particles has poly(C)-dependent RNA polymerase activity (6–8). The significance of this activity is not clear, as although 13S to 19S particles bind reovirus ssRNAs, they are unable to use them as templates for dsRNA synthesis (7).

We have recently cloned and sequenced the reovirus genomic segment encoding σNS (12, 21). To further our studies on the role of this reovirus nonstructural protein in the reovirus replication cycle, we have placed the coding region for σNS into an *Escherichia coli* expression plasmid. This approach provided a sufficient quantity of σNS to allow initial in vitro studies on its biological properties.

**MATERIALS AND METHODS**

**Bacterial strains and plasmids.** *E. coli* RRI (17) was used to propagate all plasmids. pRC23 is an expression plasmid that contains the λ p1 promoter and a consensus Shine-Dalgarno ribosomal binding site (22), with a single *Eco*RI site immediately downstream of this binding site (15). pRC23 was propagated in the presence of the low-copy-number compatible plasmid pRK248Trh (2), which carries the gene for a temperature-sensitive λ cl repressor.

**Construction of expression plasmid for reovirus nonstructural protein σNS.** The strategy used in constructing the expression plasmid for σNS is outlined in Fig. 1. Initially, a cloned cDNA copy of reovirus type 3 genomic segment S3 (12) was partially digested with *AvaiI*. After polyacrylamide gel fractionation, the purified fragment representing the 5′ end of the S3 mRNA (147 base pairs, including the GC tail), was further digested with *DdeI*, which cut 10 bases downstream of the initiating AUG triplet. Restoration of these bases to the coding region and the addition of an *Eco*RI cohesive end were achieved by ligation to a pair of synthetic complementary deoxyoligonucleotides, prepared as described previously (1, 18). After further polyacrylamide gel purification, the fragment containing the modified 5′ end (now with an *Eco*RI site immediately upstream of the initiating AUG codon) was ligated back to the fragment representing the 3′ end of the S3 mRNA (1,110 base pairs, including the GC tail), and then the reconstructed S3 gene was subcloned into pBR322 at the *Eco*RI- *Pst*I sites. After *Pst*I digestion, T4 DNA polymerase was used to form a blunt end at the *Pst*I site. *Bam*HI linkers were added, and the resulting *Eco*RI- *Bam*HI fragment was put into pRC23 at the *Eco*RI-*Bam*HI sites. This expression plasmid was designated pRC23-S3. All ligations, restriction enzyme digests, and bacterial transformations were performed essentially as described previously (17).

**Synthesis of reovirus nonstructural protein σNS in *E. coli.*** *E. coli* RRI (pRK248Trh) cells containing the pRC23-S3 expression plasmid were grown overnight at 30°C in L broth containing ampicillin (100 µg/ml) and tetracycline (15 µg/ml). These cultures were used to inoculate M9 minimal medium (17) containing ampicillin at 100 µg/ml and supplemented with either proline (100 µg/ml) and leucine (50 µg/ml) or Casamino acids (CAA). Cultures were grown at 30°C to early logarithmic phase (optical density at 600 nm, 0.2 to 0.3), divided in half, and either maintained at 30°C or transferred to a water bath at 42°C. Recombinant σNS (rσNS) synthesis...
Chromosomal DNA and cell debris were pelleted by centrifugation at 17,000 rpm for 1 h in an SW27 rotor. The supernatant was subjected to centrifugation (24,000 rpm for 6 h in an SW27 rotor) onto a 40% (wt/vol) sucrose cushion. The pellet, containing predominantly rσNS, was resuspended in lysis buffer (without lysozyme), made 0.8 M with respect to NaCl, and centrifuged at 35,000 rpm for 5 h in an SW41 rotor. The salt concentration of the supernatant containing rσNS was reduced to less than 25 mM by buffer exchange with TEM buffer (25 mM Tris [pH 7.5], 1 mM EDTA, 1 mM 2-mercaptoethanol) in an ultrafiltration cell (Amicon Corp., Danvers, Mass.; YM10 membrane). The supernatant was then applied to a poly(A)-agarose column [AG-poly(A) type 6; column size, 0.6 by 3 cm; Pharmacia, Inc., Piscataway, N.J.] and washed extensively with 0.1 M NaCl-TEM buffer followed by 0.2 M NaCl-TEM buffer; the bound rσNS was eluted with 0.5 M NaCl-TEM buffer. The eluted fraction, after buffer exchange (to 25 mM Tris [pH 7.5], 1 mM EDTA, 1 mM dithiothreitol, 30% glycerol) and concentration in the Amicon ultrafiltration cell, was used in all nucleic acid-binding experiments.

**Peptide mapping with Staphylococcus aureus V8 protease.** Recombinant rσNS synthesized in E. coli and rσNS synthesized in reovirus-infected L cells were labeled with [35S]methionine and fractionated on 10% SDS-polyacylamide gels. The appropriate gel slices were excised, and peptide mapping with S. aureus V8 protease (10) was performed by the technique of Cleveland et al. (3) as modified by McCrae and Joklik (19). The digestion products were analyzed on 15% SDS-polyacrylamide gels.

**Immunoblot analysis.** Electroblotting of proteins onto nitrocellulose filters was performed in a Trans-Blot electrophoretic cell (Bio-Rad Laboratories, Richmond, Calif.) essentially as described previously (27). The transfer buffer was 25 mM Tris (pH 8.3)–192 mM glycine–20% methanol (vol/vol)–0.1% SDS. The transfer was run at 0.21 A for 6 h. Proteins were detected by a horseradish peroxidase enzyme immunoassay (9).

**Preparation of 32P-labeled single-stranded and double-stranded nucleic acids.** 32P-labeled reovirus and cytoplasmic polyhedrosis virus ssRNAs were synthesized in vitro as described previously (5). Yeast tRNA, yeast tRNA, and dsRNAs were 3’ end labeled with cytidine 3’,5’-[32P] bisphosphate (25). The pBR322 dsDNA fragment was repair labeled essentially as described by Maniatis et al. (17).

**Nitrocellulose membrane filter assay for nucleic acid binding.** 32P-labeled single-stranded and double-stranded nucleic acids, in either TE buffer (25 mM Tris [pH 7.5]–1 mM EDTA) or 0.1 M NaCl-TE buffer, were mixed with rσNS. After 15 min at 4°C, the reaction mixtures were diluted eightfold with the same buffer, and 100-μl duplicate samples were filtered through nitrocellulose filters in a 96-well minifold filtration unit (Schleicher & Schuell, Inc., Keene, N.H.). Filters were washed with 15 volumes (1.5 ml) of 0.4 M NaCl-TE buffer, dried, suspended in nonaqueous scintillation fluid (Beckman Instruments, Inc., Fullerton, Calif.), and counted in an LS7800 liquid scintillation spectrometer (Beckman Instruments). Typically, 130 to 750 ng of rσNS and 1 to 10 ng of nucleic acid were used in binding experiments. Unless indicated, the amount of nucleic acid used in experiments did not saturate the available binding sites on rσNS.

**Preparation of antibody to rσNS.** Recombinant rσNS protein was obtained by fractionation of partially purified material on a preparative 7.5% SDS-polyacrylamide gel, excising the appropriate gel slice, and homogenizing it with Freund complete adjuvant. Three New Zealand White rabbits were

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**FIG. 1.** Construction of rσNS expression plasmid. The manipulations involved in the construction of pRC23-S3 expression plasmid are outlined above and described fully in Materials and Methods. In essence, the 5’ noncoding region of the S3 gene was replaced by an EcoRI site, thus allowing the juxtaposition of the initiating AUG codon for rσNS protein with the consensus Shine-Dalgarno sequence present in pRC23. Symbols: A, AvaiI site; B, BamHI site; D, DdeI site; E, EcoRI site; P, PstI site; T4 Polym., T4 DNA polymerase. The open box depicted on pRC23 represents the λ PL promoter and consensus Shine-Dalgarno ribosomal binding site.

was detected by pulse labeling with [35S]methionine (15 to 16 μCi/150 μl of culture; ca. 1,400 Ci/mmol) in M9 minimal medium containing proline and leucine. Synthesis could also be detected by Coomassie brilliant blue staining if cultures were grown in M9 minimal medium supplemented with CAA. Cell samples (usually 150 μl) were pelleted, dissolved in sample buffer, and electrophoresed on either 10 or 12.5% sodium dodecyl sulfate (SDS)-polyacrylamide gels by the method of Laemmli (16).

**Purification of rσNS synthesized in E. coli.** RRI (pRK248clts) cells containing the pRC23-S3 expression plasmid were grown at 30°C in M9 minimal medium supplemented with CAA to an optical density of 600 nm of 0.2 to 0.3. Cultures were transferred to 42°C, and cells were collected by centrifugation (3,500 rpm for 20 min in a Sorvall RC3 rotor) 3 h later. Cell pellets were washed with 25 mM Tris (pH 7.5)–5 mM EDTA, repelleted, and store at −20°C. Cells were lysed by suspension in lysis buffer (25 mM Tris [pH 7.5], 1 mM EDTA, 0.15 M NaCl, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 5% glycerol, lysozyme at 0.2 mg/ml) with at least 70 ml/250 ml of original culture.
then each injected at multiple spots on the back with approximately 100 μg of rNS. Four weeks later rabbits were boosted with the same amount of material, and 1 week later these rabbits were bled and tested for the presence of antibody against rNS by immunoblot analysis (9, 27). Antibody directed against all reovirus-specific proteins was kindly provided by N. Ikegami (Osaka National Hospital, Osaka, Japan) and was prepared from rabbits inoculated with reovirus-infected MA104 cells.

Materials. All radioisotopes were from Amersham Corp., Arlington Heights, Ill. Ribonucleoside triphosphates and poly(A)-agarose were purchased from P-L Biochemicals, Inc., Milwaukee, Wis. All enzymes used for molecular biology were obtained from either Amersham Corp., New England Biolabs, Inc., Beverly, Mass., or Bethesda Research Laboratories, Inc., Gaithersburg, Md. Reagents used for polyacrylamide gel electrophoresis and electroblotting were purchased from Bio-Rad Laboratories. Affinity-purified antibodies used for immunoblotting were purchased from Boehringer Mannheim Biochemicals, Indianapolis, Ind.

RESULTS

Construction of expression plasmid pRC23-S3. The expression plasmid pRC23, a derivative of pBR322 (15), was used to produce the reovirus nonstructural protein εNS in E. coli cells. Expression of heterologous genes in pRC23 is driven by the phage λ pLI promoter. The plasmid was designed to express inserted genes by using their own initiating AUG codons, thus allowing the production of the protein with a primary amino acid sequence identical to that of the authentic polypeptide. The pLI promoter is under the control of the temperature-sensitive cl repressor encoded by the compatible plasmid pRK248cIts (2). Hence, expression of the heterologous gene is repressed during cell growth at 30°C and induced by shifting cells to 42°C.

The recombinant plasmid pRC23-S3 was constructed as outlined in Fig. 1. Essentially, a cloned cDNA copy of reovirus genomic segment S3 (encoding rNS) was modified by replacing the 5′ noncoding region with an EcoRI cohesive end. This was accomplished by making use of a conveniently located DdeI site and a pair of synthetic complementary deoxynucleotides. To enable correct insertion to be made into pRC23, a BamHI site was added to the 3′ end. Ligation to EcoRI-BamHI-digested pRC23 resulted in the location of the initiating AUG for εNS immediately downstream of the consensus ribosomal binding site (22) engineered into pRC23 (15).

Synthesis of εNS in E. coli RR1(pRK248cIts) cells transformed with pRC23-S3 was detected by pulse labeling with [35S]methionine followed by SDS-polyacrylamide gel electrophoresis and autoradiography (Fig. 2). In the three transformants examined, incubation at 42°C resulted in the synthesis of a protein of approximate molecular weight 41,000 (lanes 2, 4, and 6). The induced protein comigrated with εNS synthesized in reovirus-infected L cells (see Fig. 5) and was designated recombinant εNS (rNS). rNS was detected within 5 min of shifting the cultures to 42°C and was still being synthesized 6 h after induction (Fig. 3A). In a pulse chase experiment, rNS labeled 30 min after cultures were transferred to 42°C and then chased with cold methionine proved to be quite stable, still being detectable 6 h after labeling (Fig. 3B). As might be expected from these results, the presence of rNS in E. coli extracts could be detected by staining SDS-polyacrylamide gels with Coomassie brilliant blue (Fig. 3C). Maximum accumulation of rNS occurred by

FIG. 2. Synthesis of rNS in E. coli. Overnight cultures of three transformants picked at random were used to inoculate M9 minimal medium supplemented with proline (100 μg/ml) and leucine (50 μg/ml). Cultured cells were pulse labeled, pelleted, and dissolved in sample buffer, and the pattern of protein synthesis was analyzed by 10% SDS-polyacrylamide gel electrophoresis and autoradiography. Lanes 1, 3, and 5, 30°C controls for the three transformants used in this experiment: lanes 2, 4, and 6, 42°C induced cultures of the three transformants. The arrow shows the position of rNS. The molecular weight markers (Bio-Rad Laboratories) were bovine serum albumin (68,000), ovalbumin (45,000), carbonic anhydrase (31,000), and soybean trypsin inhibitor (21,000).

3 h postinduction, at which time rNS constituted 6 to 7% of total cellular protein. This time was chosen for large-scale production of rNS.

Confirmation of rNS identity. The authenticity of rNS produced in E. coli cells was examined in two ways: (i) by partial proteolytic digestions with S. aureus V8 protease, and (ii) by reactivity with antibody in immunoblots. S. aureus V8 protease digests of rNS and authentic εNS obtained from reovirus-infected L cells indicated that the two proteins were, by this criterion, identical (Fig. 4). In immunoblots, rabbit antibodies raised against rNS reacted with authentic εNS synthesized in infected L cells (Fig. 5A). Also, rabbit antibodies raised against reovirus-infected cells (and, hence, recognizing most reovirus-specific proteins) reacted with rNS (Fig. 5B). These results provided further evidence that rNS was similar, if not identical, to the authentic εNS produced in virus-infected cells.

Purification of rNS produced in E. coli cells. Cells containing the expression plasmid pRC23-S3 were grown and induced in M9 minimal medium containing CAA as described in Materials and Methods. After cell lysis and pelleting of cell debris and chromosomal DNA, the supernatant (Fig. 6, lane 1) was subjected to further centrifugation at 24,000 rpm to pellet rNS. Recombinant εNS was solubilized by suspension in high-salt buffer, and insoluble contaminants were
pelleted by centrifugation at 35,000 rpm. The supernatant (Fig. 6, lane 2), after buffer exchange to lower the salt concentration, was subjected to poly(A)-agarose column chromatography as described in Materials and Methods. Recombinant σNS was purified by this procedure to virtual homogeneity as judged by SDS-polyacrylamide gel electrophoresis and silver staining (Fig. 6, lane 3). This fraction was used for subsequent nucleic acid-binding experiments.

**Preliminary characterization of the nucleic acid-binding properties of rσNS.** Initially, the ability of rσNS to bind reovirus mRNAs that were methylated and capped was compared with its ability to bind uncapped mRNAs. Accordingly, 32P-labeled reovirus ssRNAs synthesized in vitro in either the presence or absence of S-adenosyl methionine (5) were mixed with purified rσNS, and binding affinities were determined in a membrane filter assay, as described in Materials and Methods. Recombinant σNS bound both mRNAs with equal efficiency, with more than 90% of the 32P-labeled input mRNAs being retained on the filter (Table 1), suggesting that the 5' cap structure was not necessary for binding. A similarly purified fraction from noninduced E. coli cultures did not bind either of the mRNAs. Neither did the fraction from induced cultures that eluted with low salt from the poly(A)-agarose column, even though at least 50% of rσNS loaded on the column was found in this fraction (Table 1). This result indicated that not all rσNS proteins were capable of binding ssRNAs, a characteristic also reported for σNS obtained from reovirus-infected L cells (7). It should be pointed out that native rσNS protein was essential for binding, as boiling rσNS for 5 min drastically reduced binding activity (by 90%; data not included). Hence, a nonspecific aggregation of rσNS and reovirus mRNAs appears to be ruled out.

FIG. 3. Characteristics of rσNS production in E. coli cells. (A) Time course of production. A culture of E. coli RRl(pRK248 cIzs) cells containing pRC23-S3 was grown as described in Materials and Methods. Aliquots were pulse labeled and analyzed as described in the legend to Fig. 2. Lanes: 1, 30°C control; 2 through 7, 42°C induced culture at 10, 30, 60, 120, 240, and 360 min, respectively, after transfer. The arrowhead indicates the position of rσNS. (B) Stability of rσNS. Cultures were grown and induced as described above. At 30 min after transfer to 42°C, cells (3 ml) were pulse labeled for 2 min with [35S]methionine (300 μCi) and then chased with cold methionine (3 mg/ml). Portions (150 μl) were taken at 0, 10, 30, 60, 120, 240, and 360 min after labeling (lanes 2 through 8, respectively). Lane 1 shows a control. (C) Accumulation of rσNS in E. coli cells. Cultures were grown at 30°C in M9 minimal medium supplemented with CAA (instead of proline and leucine) to an optical density at 600 nm of 0.2 to 0.3 and then transferred to 42°C. Samples (150 μl) were taken at 1, 2, 3, and 6 h after transfer (lanes 2 through 5, respectively), subjected to 10% SDS-polyacrylamide gel electrophoresis, and stained with Coomassie brilliant blue. Lane 1 is a 30°C control.

![Image](http://jvi.asm.org/)
The specificity of rσNS binding was examined next. Recombinant σNS was mixed, as described above, with either reovirus ssRNA, cytoplasmic polyhedrosis virus (CPV) ssRNA, reovirus dsRNA, or CPV dsRNA. Neither of the 32P-labeled dsRNAs was retained on the filter (Table 2), demonstrating that rσNS, like authentic σNS from infected L cells, had low or no affinity for dsRNAs (11). Both ssRNAs were bound, apparently with equal efficiency (Table 2). This study was extended by examining the ability of rσNS to bind yeast tRNA, yeast RNA, and native and denatured dsDNA (a pBR322 fragment). While the overall percentage of input nucleic acid retained on filters was less than that in previous experiments, the results were the same (Table 2). Thus, the three ssRNAs (reovirus, CPV, and yeast rRNAs) were bound to approximately the same extent, whereas the two dsRNAs (reovirus and CPV RNAs) were not bound at all. While dsDNA was not retained on filters, consistent with the previously characterized σNS from infected L cells (11), denatured dsDNA was retained, although not to the same extent as ssRNAs. Retention of yeast tRNA, which has extensive secondary structure, was low (Table 2).

The number of available binding sites was estimated in competition experiments with unlabeled reovirus ssRNAs. For approximately 130 ng of rσNS, 10 to 15 ng of competing ssRNA was sufficient to cause a significant reduction in the binding of 32P-labeled reovirus ssRNA (data not shown). From this it could be estimated that the molar ratio of rσNS to bound ssRNA was approximately 150:1. This was close to that estimated for σNS from infected L cells (26).

**Inhibition of binding by GTP.** In a preliminary experiment it was noticed that the capacity of rσNS to bind ssRNAs was abolished if all four ribonucleotides were included in the reaction mixture. The ability of each of the four ribonucleotides (ATP, CTP, GTP, and UTP) to inhibit binding was then examined. GTP alone proved able to reduce binding to the extent observed when all four ribonucleotides were included (percent ssRNA retained on the membrane in

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**TABLE 1. Binding of reovirus ssRNAs by rσNS**

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<th>Conditions</th>
<th>% of ssRNAs retained on membrane</th>
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<tr>
<td></td>
<td>42°C induced cultures</td>
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<tr>
<td>poly(A)-agarose eluted*</td>
<td>30°C noninduced cultures</td>
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<tr>
<td>High salt fraction</td>
<td>Low salt fraction</td>
</tr>
<tr>
<td>S-Adenosyl methionine present</td>
<td>94</td>
</tr>
<tr>
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*Conditions used as described in Materials and Methods.
* Obtained as described in Materials and Methods.
the first experiment was 61, 58, 5, 59, and 5% of the control value for 2 mM ATP, 2 mM CTP, 2 mM GTP, 2 mM UTP, and a mixture of all four at 2 mM each. With the amount of rUNS used in these experiments (130 ng per reaction), GTP inhibition was evident between 0.5 and 1 mM (percent ssRNA retained was 93, 95, 29, 15, and 0% of control value for 0.1, 0.5, 1, 2, and 10 mM GTP, respectively). The other three ribonucleotides caused little interference with binding even at concentrations of 10 mM (percent ssRNA retained was 95, 81, and 66% of control value for 10 mM ATP, 10 mM CTP, and 10 mM UTP, respectively).

**DISCUSSION**

We have constructed an expression plasmid that synthesizes the reovirus nonstructural protein σNS in *E. coli*. The expressed protein, rUNS, and authentic σNS obtained from reovirus-infected L cells were indistinguishable when compared by SDS-polyacrylamide gel electrophoresis, partial *S. aureus* V8 protease digestion, and immunoblot analysis with antibodies raised against either rUNS or reovirus-infected mammalian cells. In addition, many of the nucleic acid-binding properties of rUNS reported here were the same as those previously reported for authentic σNS (7, 11), in particular, the affinity of rUNS for ssRNAs and the total lack of binding to dsRNAs and dsDNAs. Recombinant σNS showed no preference for reovirus ssRNAs over other heterologous ssRNAs such as CPV mRNA and yeast rRNA. Authentic σNS showed a similar lack of preference, also binding heterologous ssRNAs (7, 11). Finally, the number of rUNS proteins bound by reovirus ssRNAs was estimated to be similar to that reported for authentic σNS. However, one difference noted between rUNS and the 13S to 19S particles studied by Gomatos et al. (6–8) was that rUNS, unlike 13S to 19S particles, does not require the presence of divalent cations to bind ssRNAs. Despite this, the overall similarities between rUNS and authentic σNS suggest that the σNS synthesized in *E. coli* cells is virtually the same as that produced in infected mammalian cells.

Purification of rUNS from *E. coli* cells was simple and rapid, facilitated in part by the formation of cells of rUNS complexes that could be dissociated by high salt concentrations. This situation differs somewhat from that observed with other proteins expressed in *E. coli* cells. For instance, the H- ras gene p21 protein product remains in the insoluble membrane fraction after cell lysis and centrifugation (15). Solubilization of p21 required 3 M guanidine hydrochloride. This difference between p21 and rUNS complexes probably relates to the nature of the complex components. Recombinant σNS found in *E. coli* is probably associated with host cell ssRNAs, as many of the characteristics of rUNS complexes were similar to previously defined σNS complexes from infected L cells (11). The association between rUNS and cell ssRNAs may have prevented rUNS from entering into insoluble protein inclusion bodies.

The affinity of σNS for ssRNAs suggests that its prime role during reovirus replication is to bind ssRNAs, presumably reovirus mRNAs. However, if σNS is involved in the selection and condensation of 10 different reovirus ssRNAs, a greater specificity for reovirus mRNAs might be expected. This is not the case for either purified σNS or purified rUNS, both binding a variety of heterologous ssRNAs. An additional factor, either reovirus specific or of host origin, may be required to interact with σNS for specificity.

An interesting feature of rUNS binding of ssRNAs was the inhibition observed with GTP. A large molar excess of GTP was required, indicating that the affinity of rUNS for GTP was relatively low. Whether GTP inhibition results from competition with ssRNAs for the same site, or whether GTP binds to a second site, thus inducing a conformation change in the ssRNA binding site, remains to be resolved. GTP binding may be related to the poly(C)-dependent poly(G) polymerase activity of 13S to 19S particles (6–8). In our preliminary experiments, however, no poly(C)-dependent poly(G) polymerase activity was detected in purified rUNS preparations.

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**LITERATURE CITED**


