Bovine rotavirus segment 5 protein expressed in the baculovirus system interacts with zinc and RNA

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The cDNA sequence of genomic segment 5 of bovine rotavirus (RF strain) has been inserted into baculovirus transfer vectors, downstream of the polyhedrin promoter. Recombinant baculoviruses containing gene 5 were selected and the protein was expressed to high yields in Spodoptera frugiperda cells. The recombinant protein was inoculated into rabbits and mice to produce specific hyperimmune antisera. The polyclonal antisera reacted with a protein in rotavirus-infected MA104 cells and with a protein translated in vitro. This serum was also used to confirm that the gene 5 protein is not a structural protein. Recently, the gene 5 product has been predicted to be a zinc finger protein and reported to contain a highly conserved arrangement of cysteine residues; here, we demonstrate that the recombinant gene 5 protein binds zinc and is an RNA-binding protein as are several other zinc finger proteins.

Introduction

The rotavirus protein encoded by RNA segment 5 (NS53, NcVP2 or VP5) is generally considered to be a non-structural protein despite some different results (McCrae & McCorquodale, 1982). The gene encoding this protein belongs to a group of four genes (genes 5, 6, 7 and 9), called early genes, whose transcription is independent of host protein synthesis in infected cells (Johnson & McCrae, 1989). The early pattern of viral transcription suggests that some of the products of these four genes may play a regulatory role in the viral replication cycle. Gene 5 protein is expressed at a very low level in infected cells and has been detected in precore complexes which appear at a very early stage of virus particle assembly (Gallegos & Patton, 1989). Although the gene 5 protein has been expressed in Escherichia coli and sequenced (Brémont et al., 1987), its precise biochemical structure and functional role in the replication of rotaviruses are not yet known (Estes & Cohen, 1989).

Amino acid sequence analysis revealed that the gene 5 protein is relatively rich in cysteine residues and has two sequence elements with an arrangement of cysteines and histidines characteristic of zinc fingers. The first motif corresponding to the sequence (Cys-X2-Cys-X5-Cys-X2-Cys) where X indicates any other amino acid (aa) is present in the amino-terminal region (residues 53 to 69). The second motif (His-X2-Cys-X6-Cys-X2-Cys) maps between aa 315 and 328. In contrast to findings with other rotavirus structural and non-structural proteins that are highly conserved between group A isolates, rotavirus gene 5 shows extensive sequence diversity (Mitchell & Both, 1990). However the first motif is conserved throughout all the group A strains sequenced so far (Xu et al., 1990) as well as in the porcine group C strain (Brémont et al., 1990). These observations suggest an important role of the cysteine-rich region in protein function. First identified in transcription factor TFIIIA (Miller et al., 1985), zinc finger domains are a frequent feature of many regulatory and nucleic acid-binding proteins (Evans & Hollenberg, 1988).

The possibility of the presence of a zinc finger and the interaction of the gene 5 protein with nucleic acids was investigated by using a recombinant gene 5 protein expressed to high levels in the baculovirus system.

Methods

Viruses and cells. The RF strain of bovine rotavirus was propagated in foetal rhesus monkey kidney (MA104) cells, as described previously (L'Haridon & Scherrer, 1976). Autographa californica nuclear polyhedrosis virus (AcNPV) and recombinant virus stocks were grown and assayed in confluent monolayers of Spodoptera frugiperda cells in Hink's medium containing 10% foetal bovine serum (FBS) according to procedures described previously (Summers & Smith, 1987). Light (L) and dense (D) particles were purified as described (Bican et al., 1982).

DNA manipulation and recombinant virus construction. The full-length clone pRF5 (Brémont et al., 1987), was digested with NcoI and HpaII
and subcloned either into the Smal site of the baculovirus transfer vector pAC461 or in the BamHI site of the transfer vector pVL941 filled in with the Klenow fragment (Luckow & Summers, 1988). Recombinant baculoviruses were obtained and screened as previously described (Cohen et al., 1989). For each transfer vector, three independent recombinant viruses designated pAC461/RF5.1 to pAC461/RF5.3 and pVL941/RF5.1 to pVL941/RF5.3 were prepared. A transcription vector containing the entire genome segment 5 cloned in pBS+ (Stratagene) was constructed by inserting the pRF5 DNA fragment containing the gene 5 open reading frame into the Smal site. The recombinant plasmid pBS/RF5 was linearized with EcoRI and transcribed using T3 RNA polymerase.

Preparation of radiolabelled cell extracts. Monolayers of S. frugiperda cells (6 × 10⁶) in tissue culture flasks (75 cm²) were infected with recombinant viruses at a multiplicity of 10 p.f.u./cell. After a 1 h adsorption period, the inoculum was removed and 12 ml of Hink’s medium was added. Cells were incubated at 25 °C and pulse-labelled at various times after infection for 2 h with [35S]methionine (20 μCi/ml). Cells were washed with MEM, solubilized with RIPA buffer (10 mM-Tris–HCl pH 7.4, 150 mM-NaCl, 1 mM-EDTA, 1% Triton X-100, 0.5% sodium deoxycholate (DOC), 0.1% SDS and 1 μg/ml aprotinin), and sonicated for 1 min.

MA104 cells were grown in Eagle’s MEM in 75 cm² tissue flasks and infected with bovine rotavirus at high m.o.i., in the presence of 1 μg/ml trypsin (Sigma type IX). Four hours after infection, the cells were lysed in RIPA buffer.

Preparation of antiserum to the expressed gene 5 protein. Monolayers of S. frugiperda cells (2 × 10⁷ cells) were infected with recombinant pVL941/RF5.1 (m.o.i. 10 p.f.u./cell) or wild-type baculovirus at the same m.o.i. Three days p.i., cells were scraped with a rubber policeman and washed in serum-free medium. Cell pellets were obtained by low-speed centrifugation (1500 r.p.m. for 10 min) and resuspended in 1 ml of 20 mM-Tris–HCl pH 7.5, 1% DOC and sonicated at 0 °C for 30 s. Lysed cells were then centrifuged at 35000 r.p.m. for 3 min at 4 °C. The pellet was resuspended in 2 ml of buffer A (50 mM-piperidine, 5 M-urea, 1% Brij 35, 2% betaine, 10 mM-DTT pH 11-35), sonicated as above and centrifuged for 10 min at 35000 r.p.m. The supernatant was loaded on a Mono Q anion exchange column (5 × 50; Pharmacia) and the proteins were eluted with a gradient of 0 to 1 M-NaCl in buffer A. Fractions containing the rotavirus protein were pooled and dialysed at room temperature against 50 mM-Tris–HCl pH 8.6, 10% glycerol, 5 mM-reduced glutathione, 0.5 mM-oxidized glutathione.

Preparation of antisera to the expressed gene 5 protein. Hyperimmune sera were generated in four New Zealand white rabbits and 14 Balb/C or C3H mice injected intradermally at multiple sites (Waitukaitis et al., 1971) with a crude lysate of S. frugiperda cells (2 × 10⁷ cells) infected with the recombinant pVL941/RF5.1, emulsified in complete Freund’s adjuvant. Control sera were obtained from each animal before immunization. Booster intradermal injections were administered at 15, 30 and 45 days, after primary immunization. Sera were collected 3 days after the last injection and were tested for the presence of antibodies by immunoprecipitation and Western blotting with wild-type and pVL941/RF5.1 baculovirus-infected S. frugiperda cell extracts.

Immunoprecipitation and immunoblotting analysis. Extracts of S. frugiperda and MA104 cells prepared as described above were clarified by centrifugation for 20 min at 35000 r.p.m. in the Beckman TL100 ultracentrifuge. Aliquots (10 μl) of supernatants corresponding to 10⁶ MA104 cells were diluted in 80 μl of RIPA buffer. Then 10 μl of the appropriate antiserum diluted in RIPA buffer was added and incubated at 37 °C for 1 h. To these samples, 40 μl of Protein A-Sepharose beads pre-swollen in RIPA buffer was added and incubated for 1 h at room temperature. After centrifugation and three washes of the beads, proteins were solubilized by boiling for 10 min in dissociation buffer (Laemmli, 1970). Supernatants were analysed by electrophoresis on 10% SDS–polyacrylamide gels.

Proteins separated by SDS–PAGE were transferred onto Pro Blott sheets (Applied Biosystems) at constant current of 170 mA for 1 h. The transfer buffer consisted of 100 mM-3-[cyclohexylaminol]-1-propanesulfonic acid pH 11 and 10% methanol. In some experiments, blots onto nitrocellulose were produced as described by Towbin et al. (1979).

For immunostaining, the filters were soaked for 1 h in blocking buffer (3% BSA in 20 mM-Tris–HCl pH 7.4, 150 mM-NaCl); then rabbit anti-gene 5 protein antiserum diluted in blocking buffer was added and filters were incubated for 1 h at 37 °C. After washes in 20 mM-Tris–HCl pH 7.4, 150 mM-NaCl, filters were returned for 60 min to blocking buffer containing a 1:1000 dilution of alkaline phosphatase-conjugated goat anti-rabbit IgG (Bio-Isis). After further washings, staining was done using a BlueGene kit (Gibco/BRL) according to the manufacturer’s instructions.

Zinc binding assays. Radiolabelled [65Zn]Cl₂ (2.9 mCi/mg) was purchased from New England Nuclear and used to overlay a protein blot prepared as described above. Blots were soaked at room temperature for 1 h in binding buffer (100 mM-Tris–HCl pH 7.5, 5 mM-NaCl) and 1 h in binding buffer, containing [65Zn]Cl₂ (1 μCi/ml). Unbound [65Zn]Cl₂ was removed by washing twice in binding buffer and once with distilled water. For competition experiments, divalent ions were added to binding buffer, in both the binding and washing steps. Sheets were dried on filter paper and autoradiographed overnight.

RNA binding analysis. Rotavirus-specific ssRNA probes were prepared in vitro by using single-shelled rotavirus particles as described previously (Cohen, 1977) using [32P]UTP as the labelled precursor. Single-stranded RNAs were separated from dsRNAs by precipitation with 2 M-LiCl. Non-specific RNA probes were synthesized from cloned DNA inserted into a pBS vector (Stratagene Cloning System) between T7 and T3 promoters. Probe mixtures were deproteinized by two rounds of extraction with phenol:chloroform (1:1).

For the gel retardation assay purified fractions of recombinant protein were preincubated at 21 °C for 20 min in 20 μl of a standard binding buffer containing 150 mM-NaCl, 10% glycerol, 1 mM-EDTA, 20 mM-HEPES pH 7.5, 1 mM-DTT and 50 μg/ml of tRNA as competitor for non-specific RNA-protein complexes. Then 2 ng of [32P]-labelled ssRNA probe (3 × 10⁶ c.p.m.) was added and the samples were incubated at 4 °C for 30 min. Electrophoresis was performed in a 1% agarose gel in 0.5 × Tris-borate-EDTA as running buffer (Thomas et al., 1990). Gels were then subjected to autoradiography without drying.

Results

Construction of recombinant viruses

The complete coding region of a cDNA copy of rotavirus RNA segment 5 was inserted into transfer vectors pAC461 and pVL941. In order to reduce unnecessary sequences downstream of the polyhedrin promoter we used the restriction enzyme NcoI whose site includes the translation initiation ATG codon. The orientation of the gene and its junction with the transfer vector were confirmed by restriction analysis. The correct junctions.
reconstruct BamHI and NcoI sites for the vector pAC461 and the BamHI site for pVL941. The presence of gene 5 in the recombinant virus was proven by Southern blot analysis of viral DNA (data not shown).

Expression of rotavirus gene 5 in S. frugiperda cells

To demonstrate that the gene 5 protein was synthesized in recombinant baculovirus-infected S. frugiperda cells, we prepared protein extracts from S. frugiperda cells infected with pAC461- and pVL941-derived recombinants as described in Methods. Extracts were also made from AcNPV-infected and mock-infected cells. A sample of each preparation was analysed by SDS-PAGE and the proteins were stained with Coomassie blue. In comparison with the wild-type virus, recombinant viruses synthesized a unique protein with an Mr of 58K (Fig. 1), similar in size to that predicted from the amino acid sequence of the gene 5 product. The level of gene 5 protein synthesis by the pVL941-derived recombinant was five- to 10-fold greater than that from viruses derived from pAC461. pVL941/RF5.1 was used for the subsequent studies. Pulse-labelling experiments indicate that synthesis is maximum at 48 h post-infection and continues up to 72 h with no apparent degradation.

Purification of NS53 recombinant protein

As recombinant NS53 was essentially non-soluble, a variety of extraction conditions were investigated to establish conditions for recovering NS53 protein in a form suitable for purification. We found that most of the protein could be recovered in a soluble form by extraction with 5 M-urea at high pH. We also took advantage of the low solubility of NS53 to eliminate a majority of cellular proteins by treatment of Sf9 cells with 1% DOC as described in the Methods section. A typical PAGE analysis of NS53 elution is shown in Fig. 2(a). The protein was not present in the flowthrough, but was detected in fractions 40 to 42 corresponding approximately to 350 mM-NaCl and was estimated to be 95% pure by SDS–PAGE. To confirm the specificity of purified NS53 protein in the RNA retardation assay, S. frugiperda cells infected with the wild-type baculovirus were extracted and chromatographed using an identical procedure. The corresponding fractions (38 to 43) were
analysed by SDS-PAGE and shown to contain polyhedrin.

Characterization of antisera against the expressed gene 5 protein

To confirm the characterization of the recombinant protein, a hyperimmune antiserum raised to recombinant baculovirus-infected S. frugiperda cells has been used to identify gene 5 protein in rotavirus-infected MA104 cells. Inoculation of several mice and rabbits with large amounts of the recombinant protein as a crude infected Sf9 cell lysate or as purified by preparative PAGE or FPLC failed to give reacting sera when tested for their ability to immunoprecipitate the recombinant protein. A single rabbit inoculated with a crude lysate led to a serum (L 9126) able to precipitate the recombinant protein. The specificity of the serum was confirmed with in vitro translated protein obtained from rabbit reticulocyte lysates programmed with mRNA synthesized from the transcription vector pBS-RF5 (Fig. 3a, lane 3).

Radiolabelled lysates from rotavirus-infected MA104 and from mock-infected cells were prepared. The preimmune serum and the hyperimmune sera did not react with a lysate of mock-infected MA104 (Fig. 3b, lanes 1 to 3). As shown in Fig. 3, a protein having an apparent $M_r$ of 58K was immunoprecipitated with L 9126 from infected MA104 cells (Fig. 3, lane 6). Preimmune serum did not precipitate the 58K band (Fig. 3, lane 5). It should be noted that in these MA104-infected cell lysates, VP2 and VP6 were both immunoprecipitated by gene 5 protein antiserum. This result suggests either that these polypeptides were associated with gene 5 protein in some way or that this particular rabbit had been in contact with rotavirus during the immunization period. Additional experiments using Western blotting showed that the polyclonal antiserum raised to gene 5 protein also recognized, albeit poorly, VP2 and VP6 proteins in purified rotavirus particles.

Gene 5 protein is a non-structural protein

Immunoprecipitation experiments showed that gene 5 protein antibodies reacted strongly with a major 58K band in infected MA104 cells, but also to a lesser extent with other viral proteins. The mechanism by which these minor bands were also precipitated was not identified in this study, but the antiserum was useful for determining whether the gene 5 protein was present in purified virus particles. The presence of gene 5 protein in purified double-shelled and single-shelled rotavirus particles was analysed by comparison with known amounts of purified recombinant gene 5 protein in an immunoblotting assay (Fig. 4). Immunoblotting of 25 µg of double-shelled (lane 3) and single-shelled (lane 2) rotavirus particles did not detect a band in the 58K $M_r$ range. In the same assay 40 ng (approx. 0.8 pmol) of purified recombinant protein could be detected by anti-gene 5 protein antibodies (Fig. 4a). Considering the sensitivity of immunoblotting detection of the purified recombinant protein and an approximate $M_r$ of $10^8$ for the virion, it could be calculated that there are less than three molecules of gene 5 protein per particle.
Zinc and RNA binding of rotavirus NS53

Gene 5 protein binds zinc

To provide positive evidence of zinc binding to gene 5 protein we used a method previously described for detecting zinc binding to reovirus σ3 protein (Schiff et al., 1988). Autoradiography of nitrocellulose filters probed with $[^{65}\text{Zn}]\text{Cl}_2$ (Fig. 5), shows that recombinant gene 5 protein (58K) binds with high affinity. Of the five $M_r$ markers one protein known to bind zinc could be detected. Carbonic anhydrase (30K), which coordinates one zinc atom through three histidines, binds zinc strongly under non-reducing conditions. In the same conditions proteins derived from non-infected SF9 cells or from cells infected with wild-type baculovirus did not bind zinc (data not shown). To confirm the specificity for zinc we added non-radioactive divalent ions in binding buffer for competition experiments with labelled zinc. We found that 5 mM-cadmium or magnesium did not significantly reduce $^{65}\text{Zn}$ binding and that 5 mM-cobalt competed to a small degree for the binding of labelled zinc to NS53 protein (Fig. 5). In contrast, $[^{65}\text{Zn}]\text{Cl}_2$ binding was efficiently blocked by competing non-radioactive zinc (data not shown). It could also be noted that divalent ions compete efficiently for zinc binding to other proteins transferred onto the blot. The zinc blotting technique was also applied to the proteins from rotavirus-infected MA104 cells. Whereas most of the virion proteins were detected on a nitrocellulose filter with India ink none was detectable by autoradiography of the filter probed with $[^{65}\text{Zn}]\text{Cl}_2$.

Demonstration of the RNA binding abilities of NS53

Sequence analysis shows that the gene 5 protein is rich in lysine and arginine residues. Since it also possesses a zinc finger, it may be an RNA-binding protein. To investigate further the nucleic acid binding ability of the gene 5 protein, a gel retardation binding assay was also used. In this assay the protein-bound RNA was separated from free RNA by electrophoresis in a 1% agarose gel (Fig. 6). This electrophoresis system did not allow the various mRNAs to be resolved. Incubation of $^{32}\text{P}$-labelled rotavirus transcripts with increasing amounts of purified recombinant protein (see Fig. 2, fraction no. 41) results in a slower migrating band representing the RNA–protein complexes. The electrophoretic mobility of this complex is dependent on protein concentration and all of the ssRNA probe is shifted up when a sufficient amount of protein is used in the assay. In contrast, no retardation was detected with the corresponding fractions derived from mock-purified wild-type baculovirus-infected cells (see Fig. 2, fraction no. 41). Experiments with non-rotavirus-specific RNA probes indicate that RNA–protein interaction was not RNA sequence-specific. No gel retardation was observed when DNA was substituted for RNA (data not shown).
Fig. 4. Western blot analysis of purified rotavirus. Decreasing amounts of partially purified recombinant NS53 protein (4 ng, lanes 4; 40 ng, lanes 5; 400 ng, lanes 6), 25 μg of double-shelled (lanes 3) and 25 μg of single-shelled (lanes 2) purified rotavirus particles and a lysate of infected (lanes 1) MA104 cells were separated by SDS-PAGE and electroblotted onto ProBlott membrane. Blots were then immunostained with a rabbit anti-NS53 serum diluted to 1:25000 (a), or with a total anti-rotavirus serum diluted to 10000 fold (b).

Fig. 5. Zinc binding activity and competition between divalent metals and zinc for gene 5 protein binding. Proteins from an extract of pVL941/RF5-infected Sf9 cells (corresponding to 10^6 cells) (lanes 1 to 4) and Mr marker proteins (M) were subjected to electrophoresis on a 10% SDS-PAGE gel and transferred onto nitrocellulose. Blots were then cut in identical strips and incubated in binding buffer. The Mr marker proteins (Pharmacia) consisted of phosphorylase b (94K), BSA (67K), ovalbumin (43K), carbonic anhydrase (30K), soybean trypsin inhibitor (20K) and bovine α-lactalbumin (14.4K); the amount of marker proteins was adjusted to correspond to the amount of NS53. Blotted proteins were incubated with 20 μM [65Zn]C12 in the absence (lane 4) and in the presence of 5 mM competing metals (Mg^{2+}, Cd^{2+} or Co^{2+}; lanes 1 to 3 respectively). Strips were washed in binding buffer as described in Methods and then exposed to X-ray film overnight with intensifying screens.

Fig. 6. Gel retardation assay of purified recombinant gene 5 protein. 32P-labelled ssRNA prepared by in vitro transcription was incubated with recombinant or wild-type baculovirus-purified proteins. Labelled ssRNA was incubated alone in binding buffer (lane 1), or with increasing amounts of FPLC-purified proteins obtained from lysates of wild-type (Fig. 2b, fraction no. 41; lanes 2, 4 and 6) or recombinant (Fig. 2a, fraction no. 41; lanes 3, 5 and 7) baculovirus-infected cells. In lanes 3, 5 and 7, the amount of NS53 protein was respectively 0.8, 1 and 1.6 μg. In the wild-type control (lanes 2, 4 and 6), amounts were respectively 1.5, 2 and 3 μg.

Discussion

In this study we report the isolation of a recombinant baculovirus that expresses the rotavirus gene 5 protein in infected S. frugiperda cells. Data have been presented which show that the gene 5 protein is expressed to high levels in insect cells. From Coomassie blue-stained preparations of cell extracts it was estimated that the amount of gene 5 protein in cells infected at high...
multiplicity was approximately 20% of the total stainable proteins in the cell extracts prepared at the end of the course of infection. This level is higher than the level obtained previously in E. coli (Brémont et al., 1987), presumably because the protein is less rapidly degraded when prepared from S. frugiperda cells. The availability of large-scale production of the gene 5 protein will permit further structure/function analyses to be undertaken.

Difficulties in raising a hyperimmune serum against the expressed protein probably reflect the low antigenicity of NS53 and its possible similarity to a cellular protein. The same difficulties were experienced with the protein expressed in E. coli. Nevertheless, we were able to generate a high titre of gene 5 protein antiserum to characterize the protein in infected MA104 cells and to determine whether it is a minor outer capsid protein (McCrae & McCorquodale, 1982) or a non-structural protein. Our results show clearly that the gene 5 protein is not associated with purified single- or double-shelled particles, and thus must be designated a non-structural protein, NS53.

Analysis of the amino sequence of NS53 reveals two features often found in nucleic acid-binding proteins: (i) segment 5 protein is slightly basic with a net positive charge of 9 at pH 7·0 (Brémont et al., 1987) and (ii) there are two cysteine-rich regions with the consensus zinc finger motif. In addition to the consensus cysteines and histidines there is a conservation of several amino acids in these motifs (Ala-Leu, His and Val in the large loop and Trp in the small loop at the carboxy end). These repeats suggest a duplication of the motif in the bovine RF strain, but the second zinc finger is not conserved in the SA11 strain or the porcine group C rotavirus (Mitchell & Both, 1990). In the prototype zinc finger-containing protein TFIIIA and many proteins of the same group the cysteine motif is repeated up to nine times. However, several viral proteins interacting with zinc, such as the simian virus 40 large T antigen (Loebet et al., 1989), human immunodeficiency virus tat protein (Frankel et al., 1988) or gag protein of Moloney murine leukaemia virus (Meric & Goff, 1989) have a single zinc-binding sequence element.

We have used the recombinant protein to investigate the ability of NS53 to bind nucleic acids and (or) zinc. We have shown that gene 5 protein recognizes ssRNA with high affinity in a gel retardation assay, but in a non-specific fashion since it binds both rotavirus transcripts and unrelated RNA molecules. Until now only VP2, the major protein of the core, and the gene 7 protein (NS34) have been shown to be nucleic acid-binding proteins (Boyle & Holmes, 1986). The fact that NS53 was not detected as a nucleic acid-binding protein by Boyle & Holmes (1986) could have been due to the low amount of this protein in rotavirus-infected cells.

We have also demonstrated directly by a blotting technique that gene 5 protein can bind zinc. Our data invite a comparison of NS3 with other viral proteins of the reoviridae that bind zinc and/or nucleic acids. Among these proteins only reovirus σ3 has a zinc finger and has been shown to bind zinc (Schiff et al., 1988). No typical zinc finger is present in other proteins of the reoviridae, but several proteins with cysteine–histidine-rich regions have been identified [reovirus μNS, σ2, σNS; blutongue virus (BTV) VP4, NS1, NS2; rotavirus NS35] (Roy, 1989; Wiener & Joklik, 1987; Wiener et al., 1989; Both et al., 1982). Reovirus σNS and BTV NS2 bind ssRNA in a non-specific manner. Both proteins are non-structural. It has been suggested that the ssRNA-binding activity of these proteins might play a role in morphogenesis by condensing the 10 ssRNA molecules into precursor subviral particles prior to their replication (Huismans & Joklik, 1976; Huismans et al., 1987). Such a role for rotavirus NS53 is also substantiated by results of Patton's group (Gallegos & Patton, 1989). In that work subviral particles with replicase activity were resolved by electrophoresis under non-denaturing conditions in agarose gels. NS53 is found in these subviral particles. Moreover, NS34 that binds ssRNA and dsRNA (Boyle & Holmes, 1986) is also found in these subviral particles. It might be hypothesized that, for rotaviruses, two proteins are involved in the condensation of the ssRNA instead of a single protein for BTV and reoviruses. Later in morphogenesis NS53 and NS34 are released from replicating particles (Gallegos & Patton, 1989), supporting data presented here on the non-structural and functional role of the gene 5 protein.

The authors would like to acknowledge James Hejna for critical reading of the manuscript.

References


(Received 19 February 1992; Accepted 29 April 1992)