Structure of rotavirus particle: Interaction of the inner capsid protein VP6 with the core polypeptide VP3

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The structural relationship between VP6 (inner capsid polypeptide) and the viral core was studied using chemical cross-linking with dithiobis(succinimidyl propionate). Crosslinked single shelled and reconstituted rotavirus particles, suggest the existence of a complex organization of VP6 molecules in the inner capsid and a direct interaction with the core polypeptide VP3.

The inhibition of the recovery of RNA polymerase activity associated with the reconstitution of the single shelled particle in the presence of antiVP6 monoclonal antibodies indicates that a VP6 domain between amino acids 56 and 58 seems to be important in viral transcription.

A VP6 gene temperature-sensitive mutant (ts G) carrying a mutation affecting assembly of single shelled particles was used in reconstitution experiments. The mutant was able to recover RNA polymerase activity at restrictive temperature. Wild type cores or VP6 were able to reconstitute the particle with both the mutant cores and VP6. These results suggest the existence of various steps for the assembly of single shelled particles, where the VP6-VP3 interaction seems to be important for recovery of RNA polymerase activity.

Key words: *core polypeptide VP3, inner capsid protein VP6, rotavirus particle.*

INTRODUCTION

Rotavirus, a member of *Reoviridae* family, is a major cause of intestinal gastroenteritis in young children (Cukor and Blacklow, 1984; Spencer *et al*, 1984). The rotavirions have a double shelled protein structure covering a central core of icosahedral symmetry (Estes *et al*, 1983; Prasad *et al*, 1988; Yeager *et al*,1990). The protein capsids consist of an outer protein capsid made up of two polypeptides, VP7 -a glycoprotein- and VP4 -a trypsin-sensitive precursor of VP5* and VP8*- and the inner protein capsid made only of the major viral polypeptide VP6 (Cukor and Blacklow, 1984; Estes and Cohen, 1989). The central core comprises three polypeptides (VP1, VP2, VP3) and the eleven segments of double-stranded RNA, which conform the viral genome (Bican *et al*, 1982; Novo and Esparza, 1981).

The outer protein capsid has been shown to be required only for the initial interaction with the infected cell and during viral penetration (Kaljot *et al*, 1988; Suzuki *et al*, 1985; Suzuki *et al*, 1986). Virus particles containing only the inner shell proteins, which can be isolated from infected cells, are responsible for both *in vitro* and *in vivo* transcription (Gallegos and Patton, 1989; Novo and Esparza, 1981). The single shell virus transcribed, in a conservative manner, the eleven viral genes giving rise to mRNAs products corresponding to identical copies of the plus strand of the template (Spencer and Garcia, 1984).

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Previous work has shown that the removal of VP6 from the single shelled particle by incubation with high Ca^{+2} concentration results in viral cores, which lack transcriptional activity (Bican *et al*, 1982; Sandino *et al*, 1986). Incubation of those viral cores with purified VP6, restores the RNA synthesis capability (Sandino *et al*, 1986). The recovery of the transcriptional activity is dependent on the reassociation of VP6 around the viral core, where the single shell particle is reconstituted (Bican *et al*, 1982; Sandino *et al*, 1986). The single shelled particle contains, besides VP6, all enzymatic activities required for RNA synthesis in the viral core, namely: the viral transcriptase (VP1), the guanylyltransferase (VP3) and an RNA-binding activity (VP2); but it cannot transcribe in the absence of VP6 (Kumar *et al*, 1989; Pizarro *et al*, 1991; Valenzuela *et al*, 1991). The requirement of VP6 is not well understood since the polypeptide lacks enzymatic activities associated with transcription (Bican *et al*, 1982; Clapp and Patton, 1991; Sandino *et al*, 1986). These results suggest that VP6 has a structural function, interacting with other viral core proteins including VP2, rather than an enzymatic activity involved in the process of mRNA synthesis.

The purpose of the present communication is to define the interaction of VP6 with the viral core polypeptides that could explain the VP6 requirement for viral transcription.

MATERIALS AND METHODS

Viruses and cells. SA-11 virus stocks were a gift from Dr Romilio Espejo. Fetal rhesus monkey cells (MA-104) were obtained from the Instituto de Salud Pública. The SA-11 rotavirus strains were grown by infection of MA-104 cells, using m.o.i. of 1-2, as described elsewhere (Pizarro *et al*, 1991). The tsG mutant was derived from SA-11 by chemical mutagenesis and obtained as a gift from Dr Frank Ramig (Ramig, 1982, 1983). The SA-11 tsG mutant was prepared as the wild type virus, except that the ts virus was allowed to adsorb at permissive temperature and maintained at 31°C until harvested. To label viral polypeptides at 1 h postinfection the viral inoculum was replaced by methionine-free MEM containing 5 μg/ml Actinomycin D and 20 μC/ml of ^{35}S Methionine. The virus was purified from the cell lysate, as previously described (Pizarro *et al*, 1991).

Preparation of viral particles. Single shelled particles from wild type or tsG were obtained by EDTA treatment of double shelled particles, and cores were prepared from the single shelled particles by incubation with 1.5 M $CaCl_2$, as previously described (Sandino *et al*, 1986). The biological activity of both viral particles was determined by their RNA polymerase activity (Spencer and Arias, 1981; Spencer and Garcia, 1984). Each of the virus particles, double and single shelled, and the [^{35}S]-methionine labeled SA-11 virus were tested for their in vitro transcriptional activity by analysis of the RNA products by electrophoresis on acrylamide 5% urea 8 M gels (Sandino *et al*, 1986).

RNA polymerase assay. Purified single shelled viral particles or heat treated double shelled particles were assayed for RNA polymerase by incubation in a 25 μl reaction mixture containing 150 mM Tris-HCl (pH 8.5), 10 mM $MgCl_2$, 80 mM NaCl, 0.1 mM S-adenosylmethionine, 2 mM each ATP, CTP and UTP, and 0.24 mM of α-[^{32}P] GTP (SA: 400 cpm/pmol). The mixture was incubated for 30 min at 31°C or 45°C. To analyze the transcriptional products reaction, the mixtures were either acid precipitated to determine acid insoluble radioactivity or phenol extracted, ethanol precipitated and subjected to electrophoresis in 8 M urea-polyacrylamide gels, as described elsewhere (Sandino *et al*, 1986).

Reconstitution of the single shelled virus particle in the presence of antibodies. Purified SA-11 viral cores (0.3 μg) or VP6 (0.4 μg) in 10 mM Tris-HCl buffer pH 8.5 (Buffer A) were incubated with different antibodies for 30 min at 37°C. Then, either the antibodies-cores or antibodies-VP6 complexes were incubated for 30 min at 37°C with the other fractions to reconstitute the single shelled particles, as previously described (Sandino *et al*, 1986). The reconstituted single shelled particles were purified by centrifugation for 30 min at

17,000 rpm in an Eppendorf centrifuge. The viral pellet was resuspended in buffer A and assayed for RNA polymerase activity, as described above, as a criteria for reconstitution of the single shelled particle.

In these experiments, several antibodies were used: 1) subgroup I and II anti-VP6 monoclonal antibodies kindly provided by Dr A. Kapikian (NIH-USA); 2) Anti-VP6 monoclonal antibodies obtained from Dr P. Pothier (Laboratoire de Virologie, Centre Hospitalier Regional et Universitaire de Dijon, France), named RV 50, RV 443, RV1026 (Pothier *et al*, 1987; Kohli *et al*, 1992). The specificity of the antibodies was determined by: a) their reactivity against purified chemical fragments of VP6; b) their reactivity against several partially overlapping synthetic heptapeptides generated from the amino-terminus of the VP6 molecule. The VP6 region from amino acids 56 to 59 was strongly recognized by antibody RV50; RV1026 recognized a region between amino acids 57 and 59, but with low affinity with respect to RV50. Antibody RV443 interacts with VP6 domain located from aminoacids 60 to 75 (Kohli *et al*, 1992). The amount of antibody used in each reconstitution assay was determined by a specific ELISA test, where the amount of antibody required for a complete reaction with the respective viral fraction was determined. In each case, the amount of antibody necessary to reach the plateau of the ELISA reaction with the corresponding viral fraction was defined. This amount was assumed to correspond to that necessary to react to completion with the respective viral fraction.

Cross-linking analysis. The assay consisted in the incubation of 10 μg of ^{35}S-methionine-labeled single or double shelled SA-11 virus particles previously resuspended in PBS pH 7.2, containing 1 mM $MgCl_2$ with different amounts of DSP (dithiobis (succinimidyl propionate)) (Pierce). DSP was prepared before use by dissolving 10 mg in 1 ml of DMSO. The reaction mixture was incubated for 60 min at 20°C, and DSP was added when starting the reaction and again after 30 min. Then, 10 volumes of 1 M Tris-HCl buffer pH 8.0 were added and incubation was continued for an additional 30 min to terminate the reaction. The crosslinked products were analyzed electrophoretically in a 2-D gel system. For the first dimension, the viral particles were disrupted by boiling for 5 min in the Laemmli sample buffer without 2-mercaptoethanol, and then separated by SDS-PAGE in a 12% gel. Before the second dimension, also carried out in a 12% SDS gel, each gel strip containing the viral proteins was cut and incubated in a 0.5 M Tris-HCl buffer pH 6.8 containing 2% of 2-mercaptoethanol for 30 min at 37°C, to cleave the crosslinked complexes, placed on top of a 12% SDS-gel and electrophoresed. The gel was dried and subjected to autoradiography using a X-Omat Kodak film. In the second dimension, ^{35}S methionine labeled SA-11 viral polypeptides were used as markers.

Protein determinations. Viral protein concentrations were determined using the Bio-Rad protein assay, as described previously (Bradford, 1976).

RESULTS

Chemical cross-linking of viral polypeptides.

The structural relationship between VP6 and the core polypeptides was studied using chemical cross-linking of the polypeptides. Cleavable cross-linking reagents, such as DSP, allow to determine which polypeptides are involved in the cross-linking, since the crosslinked polypeptides are dissociated by cleavage of the reagent S-S bridge and the individual mobility may be determined by their migration on PAGE (Hantula and Bamford, 1988). In SDS-PAGE, the cross-linked viral polypeptides run slower than the non cross-linked ones. The gel strip containing the cross-linked polypeptides was cut, incubated with the upper gel buffer containing 2% 2-mercaptoethanol for 10 min at 37°C, placed on top of a 12% polyacrylamide SDS-gel and electrophoresed. As molecular weight markers, [^{35}S]-methionine-labeled SA-11 polypeptides were used. Several concentrations of DSP were used and the optimum was found to be 62,5 μg/ml (data not shown). Figure 1A shows an autoradiography of the two dimensional gel

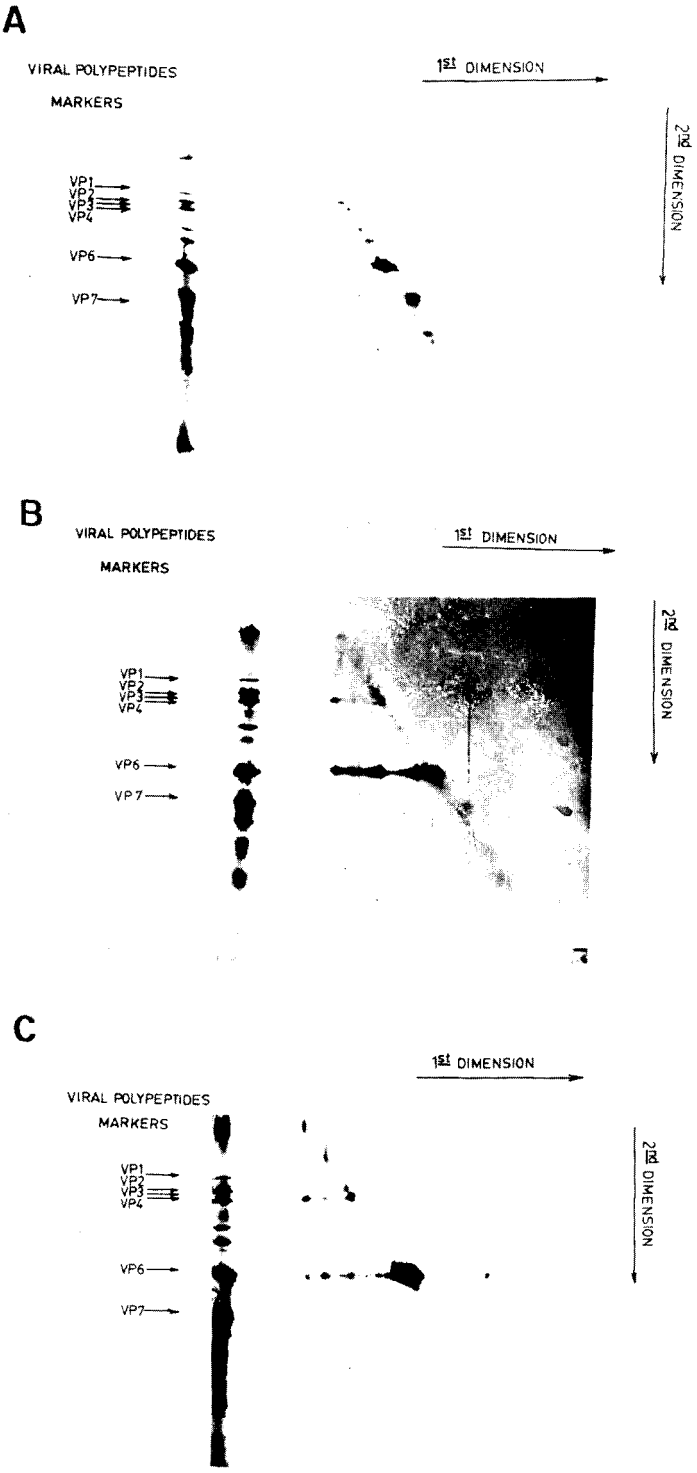

Fig 1. *Polypeptide crosslinking of rotavirus single shelled and reconstituted particles.* Single shelled ^{35}S labeled particles subjected to two dimensional gel electrophoresis in absence (A) and presence (B) of 62.5 μg of DSP. Reconstituted single shelled ^{35}S labeled particles obtained by incubation of cores and purified VP6. Particles reisolated by centrifugation and then subjected to two dimensional gel electrophoresis in presence of 62.5 μg DSP (C). In both dimension, 12% polyacrylamide gels were used. In first dimension, electrophoresis carried out in absence of 2-mercaptoethanol. In second dimension, gel strip containing viral polypeptides incubated with 2-mercaptoethanol. After electrophoresis, gel dried and subjected to radioautography. In second dimension, rotavirus S-labeled polypeptides used as markers.

from the SA-11 single shell virus without treatment with DSP. A diagonal line formed by the single shell virus proteins that migrate according to their molecular weights, as compared with the viral polypeptides marker, can be observed. Figure 1B shows an autoradiography of a two dimension gel of the SA-11 single shell virus treated with 62,5 μg/ml of DSP. The presence of the complex is detected to the left side of the diagonal line of the gel and, based on the migration in the second dimension, the polypeptides involved in the complex could be identified. As seen in the figure, some of them correspond to complexes made only of VP6 and another one corresponds to Vp6 cross-linked with the core polypeptide VP3 (as indicated by the arrow). To determine if the interaction between VP6 and VP3 is related to the recovery of RNA polymerase activity associated with the reconstitution of the single shelled particle, both purified [^{35}S]-methionine-labeled cores and VP6 were allowed to reconstitute the viral particles and the reisolated particles were subjected to a cross-linking experiment. For this, [^{35}S]-methionine-labeled SA-11 single shelled particles were treated with 1.5 M $CaCl_2$ for 20 min at 37°C, to obtain labeled viral core and VP6. After separation of cores and VP6 by centrifugation, VP6 was extensively dialyzed again in 50 mM Tris-HCl pH 7.0 buffer containing 100 mM EGTA. Subsequently, the core was incubated with VP6 protein to reconstitute the inner capsid and recover the RNA polymerase activity as previously described (Sandino *et al*, 1986). The reconstituted viral particle was then subjected to cross-linking, as described above. The results of the experiment, as shown in figure 1 C, indicate that the cross-linking proteins in the reconstituted single shell virus are the same than in single shell virus. These results support the idea that the interaction of VP6 with VP3 may be important for transcriptional activity associated with the single shelled particle. To determine if VP2 was involved in the formation of the crosslinked complexes shown above, an immunoblotting assay was carried out using anti-VP2 monoclonal antibodies. The results of such experiment were negative, suggesting that those crosslinked complexes may correspond to either VP6-VP6 or VP3- VP6, as suggested above, based on their electrophoretic mobility (data not shown).

VP6 domains involved in transcription.

To define the VP6 region involved in the interaction with the viral core that is related to the recovery of the transcriptional activity, a series of antibodies directed against different antigenic determinants present on the VP6 molecule close to the amino terminus were used. The assay consisted in the inhibition of the recovery of the RNA polymerase activity associated with the reconstitution of the single shell particle when VP6 and purified viral cores are incubated under appropriate conditions. In the assay, VP6 or the viral core are incubated with the antibodies; then, the viral core or VP6 is added, and the single shell particle is allowed to reconstitute at 37°C for 30 min. After incubation the reconstituted viral particle is reisolated by centrifugation and assayed for RNA polymerase activity by measuring ^{3}H-UMP incorporation into acid insoluble material. The recovery of the polymerase activity has been previously shown to be due to the *in vitro* reconstitution of the single shelled virus particle (Sandino *et al*, 1986; Sandino *et al*, 1988). In these experiments, the amount of antibodies used was determined by an ELISA test, in order to ensure that all the VP6 will be bound to the respective antibody before the reconstitution assay was done. The results of such experiments are shown in Table I. The RNA polymerase activity is expressed as percentage of the activity present in the single shelled particle before the preparation of VP6 and the core (lane 1). When similar amounts of purified VP6 and viral cores (prepared as described elsewhere) are incubated together, the amount of RNA polymerase activity recovered is close to 60% (lane 4) (Sandino *et al*, 1986). The viral cores were prepared by treatment with $CaCl_2$; the single shelled particle VP6 was removed until only residual RNA polymerase activity (9%) was left. A more drastic $CaCl_2$ treatment leads to the disruption of the viral core (lane 2). The instability of the viral

TABLE I

Recovery of rotavirus RNA polymerase activity after incubation with anti-VP6 antibodies

Viral fraction	Preincubation with antibodies*	Further additions	% of RNA polymerase recovery
single shelled virus 0.8 μg	–	–	100 #
core 0.3 μg	–	–	9
VP6 0.4 μg	–	–	0.1
core + VP6	–	–	56
core	anti subgroup I	–	10
VP6	"	core	53
core	anti subgroup II	–	11
VP6	"	core	60
core	anti VP6 RV50	–	13
VP6	"	core	12
core	anti VP6 RV1026	–	7
VP6	"	core	54
core	anti VP6 RV443	–	8
VP6	"	core	58

* Antibodies incubated under conditions to ensure 100% of binding to VP6.
\# 100% corresponds to 379 pmoles of [^{3}H] UMP incorporated into acid insoluble material after incubation for 30 min at 45°C.

cores makes the percentage of RNA polymerase activity associated with the reconstituted single shelled particle very variable for each viral preparation. Then, the experiment shown in Table I was done using the same viral isolates and the percentage of single shelled particles reconstituted (characteristic of the viral cores) was determined by electron microscopy, as previously described (Sandino *et al*, 1986). As seen in lane 3, there is no RNA polymerase activity associated with purified VP6. When monoclonal antibodies directed against both antigenic subgroup determinants were incubated with VP6, the recovery of the RNA polymerase activity was similar to that obtained without the addition of the antibodies. Similar results were obtained using antibodies raised against VP6 regions that were able to react with an epitope located between aminoacids 60 and 75 (antibody RV443). Also, the antibody RV 1026, directed to react with an epitope located between aminoacids 48 and 64, was unable to inhibit the recovery of RNA polymerase activity. Antibody RV 50, directed against an epitope located between aminoacids 56 and 58, was able to inhibit the reaction close to the level obtained with the viral core alone. The difference in the results obtained with different antibodies could be due to a complex structure of the VP6 molecule on that region where several epitopes could be present, as previously suggested (Pothier *et al*, 1987; Kohli *et al*, 1992). This result indicates that this VP6 region (56 to 58) is involved in the interaction with the viral core, allowing the recovery of RNA synthesis in the reconstituted single shelled particle.

Recovery of viral transcription using VP6 thermosensitive mutants.

To further explore the interaction between VP6 and the viral core, a temperature sensitive mutant carrying a mutation in VP6, which cannot assemble into single shell

particles at restrictive temperature, was used in reconstitution experiments (Mansell and Patton, 1990). The experiments were carried out using viral cores and VP6 obtained from single shelled particles of SA-11 wild type and mutant strain grown at permissive temperatures. The RNA products transcribed by reconstituted SA-11 and tsG particles are shown in Figure 2. The results show that the ts particle does not carry a mutation in the portion of the VP6 molecule required for transcription. Experiments in which the virus particles were reconstituted exchanging core and VP6 of the respective strain by the other were done. In this case, reconstitution was also carried out at 37°C and the transcription assay was done at both 45° and 31°C. When

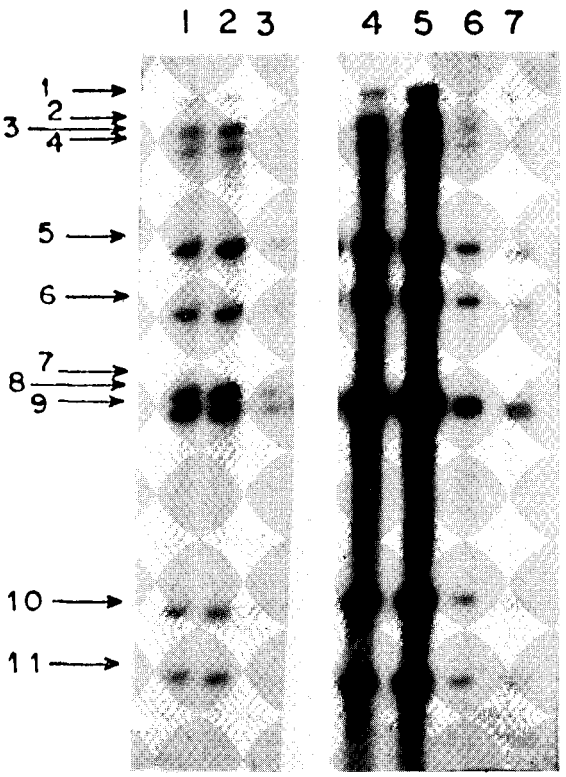

Fig 2: ***mRNA synthesis catalyzed by wild type and ts g reconstituted single shelled particles.*** Purified reconstituted single shelled SA-11 (lane 1) and tsG particles assayed for RNA polymerase activity, after reconstitution at 37°C, in reaction mixture containing α- ^{32}P-GTP, for 30 min at both 45°C (lane 2) and 31°C (lane 3). Particles reconstituted using SA-11 cores (0.3 μg) and tsG VP6 (0.4 μg) after purification by centrifugation and resuspension; particles assayed for transcription at 45°C (lane 4) and at 31°C (lane 6). Similar experiments carried out using tsB cores (0.3 μg) and SA-11 wild type VP6 (0.4 μg), after purification, assayed for transcription at 45°C (lane 5) and 31°C (lane 7).

SA-11 wild type cores and purified tsG VP6 were used for reconstitution, the amount of RNA synthesis was similar to that obtained with the homologous component (lane 4). Identical results were obtained when single shelled particles were reconstituted with tsG cores and wild type VP6 (lane 5). When the same assay was done as above, but performing the transcription assay at 31°C, recovery of the RNA polymerase activity was observed, but the amount of RNA obtained was smaller than at 45°C (lanes 6 and 7). The result suggests that the failure of the mutated VP6 molecules to bind *in vivo* at restrictive temperature to the viral core may be due to a step other than the binding to the core or the specific interaction with VP3. These results confirm that the carboxi-terminus region of VP6, where the mutation has been mapped, does not seem to be related to the recovery of transcription (Clapp and Patton, 1991).

DISCUSSION

Rotavirus mRNA synthesis seems to occur by coping the template, from the first up to the last nucleotide of the template; therefore, sequences that may act as classical promoters seem to be absent. Then, it is feasible that VP6 could be involved in a direct interaction with the template, acting as a RNA-binding protein. The possibility that VP6 may interact with the genomic RNA was studied through a series of experiments using SA-11 rotavirus, including: a) detection of viral genomic RNA-VP6 complex formation by filtration on nitrocellulose filter, using both purified [^{32}P]-labeled viral double stranded RNA and *in vitro* made mRNA and purified VP6; b) binding of [^{32}-P]-labeled genomic RNA and mRNA to VP6 previously immobilized onto nitrocellulose filters after native gel electrophoresis; c) detection of VP6 binding activity to both [^{32}P]-labeled gene 11 and to the purified transcript of the same gene by gel mobility shift assay; d) immunoprecipitation with different anti-VP6 antibodies of the VP6-RNA complex, in assays where the protein was incubated with total purified [^{32}-P]-labeled genomic RNA, and viral mRNA and VP6. The immuno-

precipitated complexes were analyzed in gel electrophoresis for the presence of genomic RNA or the transcript associated with VP6 by radioautography. In each of the assays described above, the corresponding positive controls for every step were performed. The results of those experiments showed no evidence of any kind of complex formation between VP6 and the genomic RNA or the transcripts (data not shown). This indicates that the requirement for VP6 during transcription is related to a structural constrain of transcribing a specific interaction with the viral core polypeptides by a single shelled particle.

To show if the interaction between the core and VP6 was only due to the affinity of VP6 to a unique core protein or to the complete core structure, chemical cross-linking was used. The results shown in figure 1 indicate the formation of several complexes by cross linking of structural polypeptides. The detection of cross-linked complexes of VP6 itself was a predictable result, since VP6 has been shown to form homotrimers in both infected cells and virions (Clapp and Patton, 1991; Gorziglia *et al*, 1985). The formation of a complex between VP6 and VP3 was surprising, because it was expected that VP6 will be cross-linked with VP2, since it has been shown that VP6 and VP2 are able to make single shelled like particles in cells transformed with baculovirus plasmids, that co-express both VP6 and VP2 (Labbe *et al*, 1991). These results do not necessarily indicate that VP2 and VP6 do not interact, since the formation of a cross-linked complex between VP2 and VP6 may be difficult to occur because of the accessibility of the reagent to such a place. During morphogenesis, the initial intermediate formed in the assembly of single shelled particles seems to be a particle called "precore RI", which contains the structural proteins VP1 and VP3, and then VP2 joins to constitute the core particle, indicating that VP2-VP6 interaction is required for the morphogenesis of the single shell particle (Clapp and Patton, 1991; Gallegos and Patton, 1989; Mansel and Patton, 1990; Patton and Gallegos, 1988). The ability of VP6 to interact with other viral proteins, such as VP7, has been also reported, but this does not necessarily mean that these particles are assembled in the same way *in vivo* (Sabara *et al*, 1987, 1991). Then, the results seem to indicate that VP6 could be inserted in the core particle interacting with VP3. This agrees with the reported arrangement of the VP6 on the single shelled viral particle, where it has been suggested that VP6 is not organized in a unique arrangement (Prasad *et al*, 1988).

The interference on the recovery of RNA polymerase activity associated with reconstitution of the single shelled particle when either the core or VP6 were preincubated with several anti-VP6 antibodies also suggests the existence of a specific site for VP6 to interact with the viral core, for the recovery of transcriptional activity. The finding that only one particular monoclonal antibody (RV50) was able to inhibit the recovery of polymerase activity indicates that a portion of VP6 between aminoacids 56 and 59 seems to be involved in the interaction with VP3 in the viral core. Other antibodies were unable to inhibit the recovery of RNA polymerase activity, suggesting the existence of a very specific interaction site. For example, antibody RV1026, which also reacts with the RV50 epitope based on results obtained in competition experiments, seems to recognize a different epitope (Kohli *et al*, 1992; Sandino *et al*, 1988).

The experiments in which ts G viral cores and VP6 were exchanged with their respective wild type proteins to restore the transcriptional activity, indicate that the mutation that is unable to permit the assembly of single shelled particles *in vivo* has altered the VP6 region involved in the restoration of transcription, *i.e.* the site for interaction with VP3. This suggests that the interaction between the core and VP6, to reconstitute the single shelled particle and restore RNA polymerase activity, may involve several steps which are more complex than just simple binding. For example, it has been shown that the domains for trimer formation and binding to core may be located in the center and proximity to the carboxyl terminus of the VP6 molecule (Clapp and Patton, 1991). The results of reconstitution experiments indicate that the mutation present in tsG could affect a

function that is not directly required for the interaction with VP3, but that is involved in the VP6 trimer formation.

It has been reported that other VP6 domains may be involved in the formation of the inner capsid and that a domain between aminoacids 251 and 397 could be important, but there is no information whether the particles made from the truncated VP6 fragments are functional in transcription assays (Clapp and Patton,1991). These results suggest that more than one VP6 domain may be involved: one close to the amino-terminus related to the interaction with VP3, required for RNA synthesis, and another in the center part of the molecule and close to the carboxy-terminus. Both should be important for the binding of VP6 to the core and then for the formation of the inner capsid.

Considering that VP3 is a guanylyl-transferase, it is difficult to relate this enzymatic activity of VP3 to the requirement of VP6 for transcription, even if both polypeptides interact. The formation of a cap structure is not a requisite for transcription, but the presence of 5-terminal cap structures on nascent RNA molecules makes more efficient the elongation of the transcripts, although transcription occurs in absence of cap formation (Spencer and Garcia, 1984). On other side, it has been suggested that VP3 *in vitro* has an ATPase and that rotavirus RNA synthesis requires a continuous ATP hydrolysis (Pizarro *et al*, 1991; Spencer and Garcia, 1984). Then, the requirement of VP6 for transcription could be due to a specific interaction that may exist between molecules of VP6 and VP3, where VP3 ATPase activity may provide -for example- a way of catalyzing the extrusion of the newly synthesized "cap" RNA.

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