

## Lack of Presence of VP2, VP6 or VP7 Transcripts in Virus Like Particles of Rotavirus

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### Abstract

**Introduction:** We previously established virus like particles (VLPs) and core like particles (CLPs) of rotavirus (RV) with VP2, VP6, and VP7 proteins using stable high-five cell line. Given the fact that these proteins have the ability to assemble together, we further aimed to examine in detail the possibility of viral transcripts to randomly package in VLPs and CLPs.

**Objective:** For this purpose, the presence of VP2, VP6 and VP7 transcripts were evaluated in VLPs and CLPs.

**Materials and Methods:** To investigate the presence of VP2 transcript in CLPs of RV and VP2, VP6 and VP7 transcripts in RV VLPs, RNAs were extracted from VLPs and CLPs purified from stable high-five cells and evaluated by Real-time Reverse Transcriptase (RT)-Polymerase Chain Reaction (PCR).

**Results:** The result indicated that the RV VLPs and CLPs were empty (VP2, VP6 and VP7 transcripts-free), showing that VLPs and CLPs cannot encapsidate RNAs in absence of other viral proteins.

**Conclusion:** Our results indicate the necessity of further viral proteins for sorting and packaging, which highlight the role of selective mechanism for RV packaging.

**Keyword:** Rotavirus; RNAs; Real-Time Polymerase Chain Reaction

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### 1. Introduction

Rotaviruses (RVs) represent a genus of the *Reoviridae* family [1-4], which are prominent causative agents of acute gastroenteritis (AGE) in children <5 years of age worldwide.[5, 6] RVs are non-enveloped particles that composed of icosahedral capsid enclosing 11 segments of double stranded (ds) RNA. The ds RNA segments encode six structural (VP1 to VP7) and six non-structural proteins (NSP1 to NSP6). The single layer particle (SLP) is formed by VP2 protein dimmers that surround VP1, VP3 and viral genome segments. SLPs are recoated by VP6 protein trimmers to form double layered particles (DLPs). VP7 proteins with protruding VP4 proteins form the most external layer of triple layer particles

(TLPs) that characterize mature infectious particles [7, 8]. During internalization, VP4 and VP7 of RV particles are lost, yielding a DLP. The DLPs containing enzymatic proteins (VP1 and VP3) that are transcriptionally active and produce numerous copies of RV positive RNAs, which act as mRNAs for protein synthesis and as template for replication.[9, 10] For packaging, nascent RV positive RNAs in viroplasm are selectively sorted into assembling SLPs. However, some RNA viruses apply a non-selective packaging mechanism by which genomic segments are randomly encapsidated into viral particles.

We have previously constructed RV core like particles (CLPs) of VP2 [11] and virus like particles (VLPs) of



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VP2/6/7 [12, 13] using stable high-five cell system. In this study, we used CLPs and VLPs to evaluate the presence of VP2, VP6 or VP7 transcripts, sorting randomly into VLPs.

## 2. Materials and Methods

### 2.1. CLPs and VLPs preparation

We have previously developed CLPs containing VP2 protein and VLPs containing VP2, VP6 and VP7 proteins of RV using a stable high-five cell system. High-five cell line stably expressing VP2 and high-five cell line stably expressing VP2, VP6 and VP7 were grown in serum-free SF900 medium (Life Technologies, Carlsbad, CA) in the presence of G418 and hygromycin antibiotics up to two weeks at 28 °C. Cell supernatants were clarified by centrifugation at 2000 x g for 15 min at 4 °C. CLPs and VLPs were then purified by ultracentrifugation sedimentation through 25% sucrose cushion at 100,000 x g for 75 min. Purified particles were finally resuspended in 1X PBS as previously described [11].

### 2.2. Real-time Reverse Transcriptase (RT) - Polymerase Chain Reaction (PCR) assay

RNAs was extracted from purified CLPs and VLPs of high-five stable cell line by boiling them for 10 min. The cDNA was synthesized from RNA using RT enzyme mix and 2x RT reaction mix (includes oligo (dT)20 and random hexamers) and one cycle at 25°C for 10 min, 50°C for 30 min and 85°C for 5 min according to SuperScript III First-Strand Synthesis SuperMix for qRT-PCR (Invitrogen, Carlsbad, California, CA) according to the manufacturer's instructions. Following cDNA synthesis, Real-time PCR was performed using primer pairs: VP2-F

5'-GAACCGAGACAGCTACCAA-3' and VP2-R 5'-ATCTGGCATTTCAGTAAGAACC-3' for VP2, VP6-F 5'-TCTGTTAGCAAATGTGACCG-3' and VP6-R 5'-TTCTAATGGAAGCTACCGTGA-3' for VP6, and VP7-F 5'-GCATACGCTAACTCTACTCA-3' and VP7-R 5'-ATACTCTTTGAAATAGACCGATC -3' for VP7. Real-time PCR was performed using the Corbett Research Rotor Gene™ (Qiagen, USA). In particular, PCR amplification reactions were performed in 25 µl reaction mixtures containing cDNA, 1X iQ Sybr Green supermix and 10 pmol of each primers. The reactions were incubated at 95 °C for 3 min, followed by 40 cycles of 95 °C for 30 s, 58 °C for 30 s and 72 °C for 2.5 min. Distilled water (DW) and VP2,VP6 and VP7-recombinant plasmids [11, 12] were used as negative and positive controls, respectively. A melting curve analysis was performed to confirm single gene-specific peaks by heating samples from 60 to 99 °C at the end of the amplification cycles. The linearity and accuracy of Real-time RT-PCR were evaluated using β-actin standard curve derived from amplifying serially diluted cDNA. These diluted cDNA were also used for intra- and inter-assay experiments [13].

## 3. Results

Prior to assess the presence of VP2, VP6 and VP7 transcripts in CLPs and VLPs, the linearity and accuracy of Real-time PCR were confirmed. For the intra-assay accuracy, a serially diluted cDNA was amplified with three repetitions on the same plate. The standard deviation of the mean CT value ranged from 0.07 to 0.87, with coefficients of variation < 5% (Table 1).

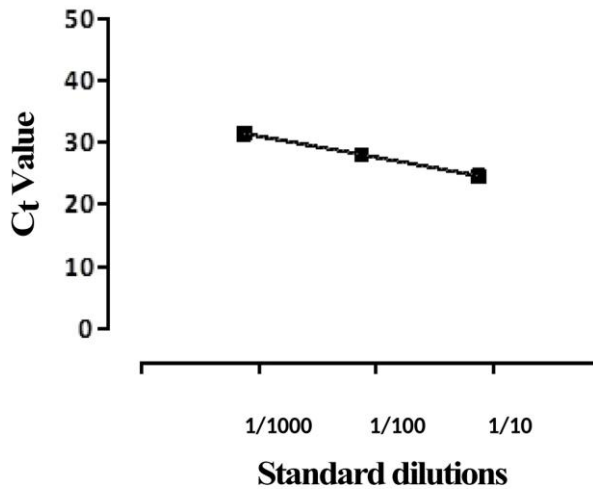
**Table 1.** Intra-assay and inter-assay reproducibility of β-actin by real time-RT-PCR

Standard Dilutions	Intra-assay for each run (three replicates)			Inter-assay for three runs		
	Mean of three replicates C <sub>T</sub> <sup>a</sup> value	SD <sup>b</sup>	CV <sup>c</sup> (%)	Mean of three runs C <sub>T</sub> <sup>a</sup> value	SD	CV(%)
1/10	25.26	0.9	3.7	25.12	0.07	0.28
1/100	28.88	0.17	0.6	28.48	0.38	1.34
1/1000	32.9	1.6	4.9	32	0.87	2.73

<sup>a</sup>Threshold cycle (C<sub>T</sub>) values

<sup>b</sup>Standard deviation

<sup>c</sup>%Coefficient of variation



**Figure 1:** The linearity range of  $\beta$ -actin real time RT-PCR. 10-fold serial dilutions of the cDNA standard starting from 1/10 to 1/1000 were amplified in triplicate by Real-time RT-PCR. The linearity and accuracy were determined by the correlation coefficient ( $R^2=1$ ) and by the equation for the standard curve ( $y = -3.415X + 34.90$ ).

To assess the inter-assay accuracy of Real-time RT-PCR, a serially diluted cDNA was amplified in three independent assays. The standard deviation of the mean CT value ranged from 0.17 to 1.6, with coefficients of variation  $< 5\%$  (Table 1.). The linearity and accuracy were determined by the correlation coefficient ( $R^2=1$ ) and by the equation for the standard curve ( $y = -3.415X + 34.90$ ) (Figure 1).

Real-time RT-PCR was used to examine the RNA content of the CLPs and VLPs. The extracted RNA was analyzed by Real-time RT-PCR and no RNA detected in samples of CLPs and VLPs produced from high-five cells.

#### 4. Discussion

Packaging and encapsidation of RV have been previously reported to be through a selective mechanism. To support this mechanism, studies proposed two models of packaging, concerted or core filling, which used for influenza A virus or  $\Phi 6$ , respectively [14]. However, there are several

problems in suggesting a core-filling model for RV [14].

In this study, we evaluated the presence of transcripts of VP2, VP6 and VP7 in CLPs and VLPs. Our results were shown that none of VP2, VP6, and VP7 transcripts could be detected within CLPs and VLPs, discarding the hypothesis that transcripts package randomly into CLPs and VLPs and suggest that sorting of RNAs is a controlled process. Moreover, previous studies have been shown that viral proteins of VP1, VP2, VP3, NSP2 and NSP5 can play critical roles in selective packaging for RV. Two proteins, NSP2 and NSP5, form dense inclusion bodies as viroplasm, which are important for sorting, packaging and replication. NSP2 acts as a molecular motor for packaging which modulate by interaction between VP2 core protein and NSP5 [15]. Moreover, analysis of early replication intermediates isolated from RV-infected cells suggested that the viral positive RNAs bind viral RNA-dependent RNA polymerase (VP1) and RNA capping enzyme (VP3), forming positive RNA/VP1/VP3 complexes that are subsequently enclosed by core protein (VP2). Consistent with this model are the high-affinity, sequence-specific and the non-specific binding activities, respectively, of VP1 and VP3 for RV positive RNAs [16-19]. Assembly of a core protein shell around VP1/VP3/+RNAs complexes may be initiated by the binding of the VP1 to the inner surface of the core through interactions with disordered N-terminal extensions protruding from the core protein. Homotypic core protein-core protein contacts are likely to be enhanced and stabilized through additional high affinity interactions of the core protein N-terminal extensions with positive RNAs, resulting in the cooperative assembly of the inner core [16-19]. The lack of trapping of the transcripts by CLPs and VLPs encapsidation in our study suggest that further viral proteins are necessary for sorting and packaging, further highlighting the role of selective mechanism for RV packaging.

#### Conflict of Interest

The authors declare that they have no conflict of interests.

## Acknowledgments

None declared.

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