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Model-based structural and functional characterization of the Rice stripe tenuivirus nucleocapsid protein interacting with viral genomic RNA

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ABSTRACT

Rice stripe tenuivirus (RSV) is a filamentous, negative-strand RNA virus causing severe diseases on rice in Asian countries. The viral particle is composed predominantly of a nucleocapsid protein (NP) and genomic RNA. However, the molecular details of how the RSV NP interacts with genomic RNA during particle assembly remain largely unknown. Here, we modeled the NP-RNA complex and show that polar amino acids within a predicted groove of NP are critical for RNA binding and protecting the RNA from RNase digestion. RSV NP formed pentamers, hexamers, heptamers, and octamers. By modeling the higher-order structures, we found that oligomer formation was driven by the N-terminal amino arm of the NP. Deletion of this arm abolished oligomerization; the N-terminally truncated NP was less able to interact with RNA and protect RNA than was the wild type. These findings afford valuable new insights into molecular mechanism of RSV NPs interacting with genomic RNA.

1. Introduction

Rice stripe virus (RSV) is a filamentous, negative-strand RNA virus belonging to the genus *Tenuivirus*. RSV can severely damage rice, the most important crop in most East Asian countries. Many outbreaks have been recorded over the last several decades (Falk and Tsai, 1998; Toriyama, 1986; Wei et al., 2009). The virus is transmitted in a circulative–propagative manner by the small brown planthopper (*Laodelphax striatellus* Fallén).

RSV contains four single-stranded genomic RNAs, designated RNA1–RNA4. RNA1 is a negative-sense segment encoding the 337-kDa RNA-dependent RNA polymerase (RdRp) (Toriyama et al., 1994). The other three genomic segments exhibit ambisense coding properties; each RNA encodes two proteins. The viral complementary RNA2 (vcRNA2) encodes the glycoprotein NSvc2 (94 kDa) (Takahashi et al., 1993; Yao et al., 2014) and the viral sense RNA2 (vRNA2) NS2 protein (22.8 kDa); these proteins suppress gene silencing and promote systemic viral movement (Takahashi et al., 1993; Zheng et al., 2015). The genomic RNA3 (vRNA3) encodes a second viral suppressor (NS3; 23.9 kDa) (Wu et al., 2014), and vcRNA3 encodes the nucleocapsid protein NP (35 kDa) (Hayano et al., 1990; Kakutani et al., 1991). The genomic RNA4 (vRNA4) encodes the disease-specific protein NS4 (20.5 kDa) (Kong et al., 2014), and vcRNA4 encodes the movement protein NSvc4 (32 kDa) (Kakutani et al., 1990; Xiong et al., 2008).

Members of the genus *Tenuivirus* exhibit close phylogenetic relationships to vertebrate viruses of the family *Bunyaviridae*, particularly the genus *Phlebovirus* (Falk and Tsai, 1998; Ramirez and Haenni, 1994). Recently, the NP crystal structures of several members of the genus *Phlebovirus* have been determined (Jiao et al., 2013; Olal et al., 2014; Raymond et al., 2012). In all bunyaviruses, the NPs are the major proteins involved in assembly of viral genomic RNAs into ribonucleoprotein complexes (RNPs). These complexes, not the naked RNAs, serve as templates for the viral RdRp, thus playing pivotal roles in both transcription and replication. However, little is known about how the RSV NP interacts with genomic RNAs or how it oligomerizes during RNP assembly. Recent studies have made some progress toward the identification of NP domains required for RNA binding and oligomerization (Cho et al., 2013; Lian et al., 2014; Zhao et al., 2015b), but the molecular details of how the RSV NP interacts with the genomic RNAs remain largely unknown.

Here, we used model-based structural and biochemical analyses to characterize the interaction of the RSV NP with RNA. We identified

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RNA-binding sites on the NP and observed that genomic RNA was sequestered in a narrow, hydrophobic binding groove. Using model-based structural predictions and biochemical analyses, we identified and mapped RNA-binding sites to a predicted charged groove in the RSV NP. These RNA-binding sites also important for protecting RNA from digestion of RNase. In addition, we found that the RSV NP could form higher-order oligomer including pentamers, hexamers, heptamers, and octamers. We also built an oligomeric model of the RSV NP and found that oligomerization enhanced RNA binding affinity and RNA protection. The models facilitate functional analysis and provide new insights into the interaction of the RSV NP and RNA.

2. Results

2.1. Recombinant RSV NP binds single-stranded RNA

To analyze the solubility and RNA-binding properties of RSV NP, we fused the gene encoding the full-length NP to a 6xHis C-terminal tag and expressed the recombinant in *Escherichia coli*. SDS-PAGE analysis revealed a specific protein band of the predicted size (36 kDa). The purified recombinant protein was further characterized by Western blotting using a monoclonal antibody (1:5000 dilution) against the RSV NP (Fig. 1A). Moreover, we found that RSV NP purified from *E. coli* contained *E. coli* RNA. We ran purified NP on a 1% (w/v) agarose gel using recombinant maltose-binding protein as the negative control. As shown in Fig. 1*B*, *E. coli* cellular RNA was present in the purified NP, but was not associated with the MBP control protein.

We then removed the *E. coli* cellular RNA by treating the NP preparation with polyethyleneimine (PEI) (Fig. 1B). We used an electrophoretic mobility shift assay (EMSA) to determine whether recombinant RSV NP bound single-stranded (ss) RNA. The digoxygenin (DIG)-labeled full-length RNA transcript of RSV S4 (2157 nt) (4.5 nmol) was incubated with increasing concentrations of PEI-treated NP (Fig. 1C). The RNA band shifted as the amount of added RSV NP increased; NP–RNA complexes were gradually formed. This suggested that the RSV NP protein bound ssRNA.

2.2. Modeling of the RSV NP and NP–RNA complexes

We built a homology model of RSV NP to better understand how the protein interacted with RNA (Fig. 2A and B). The modeled structure featured four parts: an N-terminal arm (red, aa 1–47), an N-terminal domain (yellow, aa 48–149), a C-terminal domain (green, aa 150–282), and a C-terminal arm (blue, aa 283–322). The N-terminal arm, which extends from the NP core domain, formed a unique hook-like structure that we considered might contribute to NP–NP interaction. A narrow groove was evident in the middle of the two terminal domains, which might embed RNA (Fig. 2C).

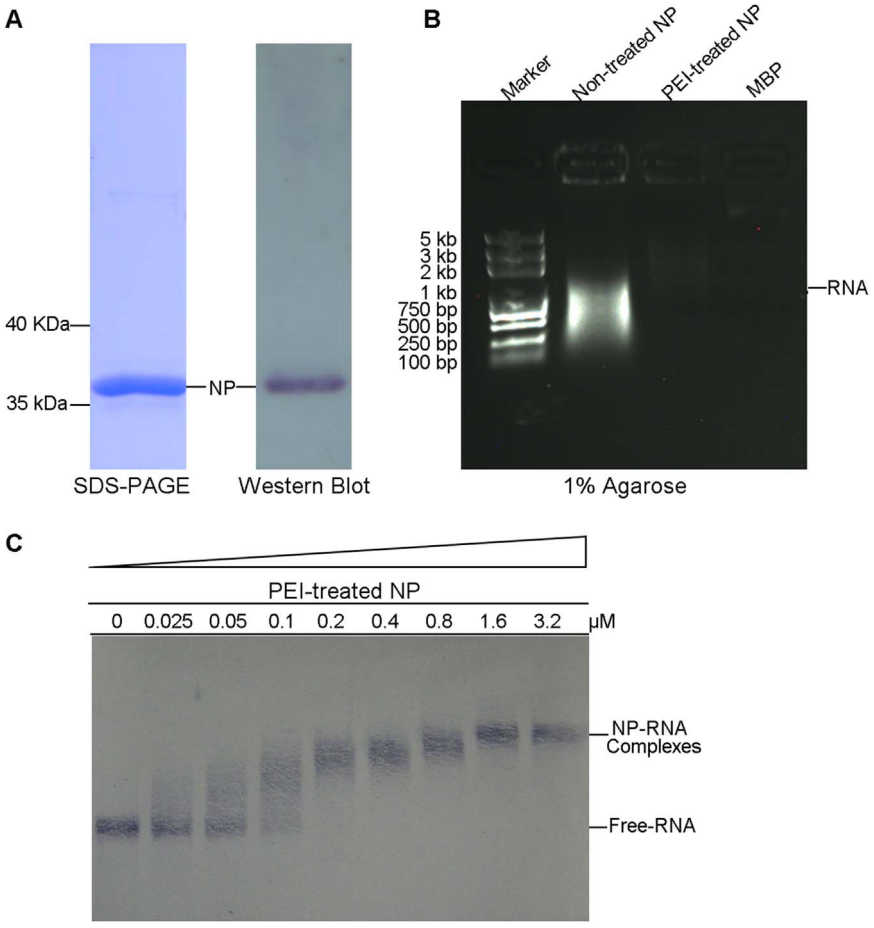

Fig. 1. RNA binding by recombinant RSV NP. (A) Expression and purification of His-tagged RSV NP protein. A 5-μg sample of purified NP was subjected to 10% (w/v) SDS-PAGE followed by staining with Coomassie Blue (left). A 100-ng sample of purified NP was detected by Western blotting using a monoclonal antibody directed against RSV NP (right). (B) Gel electrophoresis analysis of *E. coli* RNAs binding to PEI-treated or -untreated RSV NP proteins. The RNAs were electrophoresed on a 1% (w/v) agarose gel and stained with ethidium bromide. The position of the RNAs is indicated. (C) Electrophoretic mobility shift assay of RSV NP binding to genomic RNA4. Increasing amounts of PEI-treated RSV NP were incubated with 4.5 nmol of DIG-labeled RSV S4 ssRNA (2157 nt). The NP-RNA complexes were electrophoresed on a 1% (w/v) agarose gel, blotted, and visualized with the aid of an anti-DIG antibody followed by BCIP/NBT staining. The positions of free RNA and higher-order complexes are indicated.