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Identification of the strain-specifically truncated nonstructural protein 10 of porcine reproductive and respiratory syndrome virus in infected cells

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Abstract
The nonstructural protein 10 (nsp10) of porcine reproductive and respiratory syndrome virus (PRRSV) encodes for helicase which plays a vital role in viral replication. In the present study, a truncated form of nsp10, termed nsp10a, was found in PRRSV-infected cells and the production of nsp10a was strain-specific. Mass spectrometric analysis and deletion mutagenesis indicated that nsp10a may be short of about 70 amino acids in the N terminus of nsp10. Further studies by rescuing recombinant viruses showed that the Glu-69 in nsp10 was the key amino acid for nsp10a production. Finally, we demonstrated that nsp10a exerted little influence on the growth kinetics of PRRSV in vitro.

Keywords: porcine reproductive and respiratory syndrome virus (PRRSV), nonstructural protein 10 (nsp10), viral replication

1. Introduction
Porcine reproductive and respiratory syndrome (PRRS) is one of the most important viral diseases that have caused huge economic losses to the pig industry worldwide (Zhou and Yang 2010; Holtkamp et al. 2013). PRRS is mainly characterized with respiratory problems in piglets and severe reproductive illness in sows and gilts (Pejsak et al. 1997). The etiologic agent, porcine reproductive and respiratory syndrome virus (PRRSV), belongs to the Arterivirus genus of the Arteriviridae family, together with equine arteritis virus (EAV), simian hemorrhagic fever virus (SHFV) and lactate dehydrogenase-elevating virus (LDV) (Snijder et al. 2013). Given the genetic and antigenic diversity, PRRSV can be classified into two genotypes: European (genotype 1) and North American (genotype 2), which share 55–70% nucleotide identity (Meng et al. 1994; Nelsen et al. 1999). In 2006, atypical PRRS outbreak caused by the highly pathogenic PRRSV (HP-PRRSV) emerged in China, which brought fatal pathogenicity to pigs compared to previous PRRS caused by classical low pathogenic PRRSV (LP-PRRSV) strains (Tian et al. 2007; Tong et al. 2007; Zhou et al. 2009). More recently, the NADC30-like PRRSV isolates were reported (Zhou et al. 2015) and experimental animal assays revealed that importation and
recombination are responsible for the latest emergence of highly pathogenic PRRSV in China (Zhao et al. 2015). Both the North American and European PRRSV isolates are circulating in China and the HP-PRRSVs have become the dominant strains (Chen et al. 2011; Li et al. 2011; Xie et al. 2014).

PRRSV is an enveloped, positive-stranded RNA virus with a genome of about 15 kb in length, containing two large open reading frames (ORF1a and ORF1b) and several downstream ORFs (ORFs 2 to 7) (Meulenberg 2000). ORFs 2 to 7 encode the structural proteins GP2, E, GP3, GP4, GP5, M, N and the newly discovered ORF5a protein, respectively (Snijder and Meulenberg 1998; Firth et al. 2011; Johnson et al. 2011). ORF1a and ORF1b encode the viral replicate polyproteins pp1a and pp1ab, which are subsequently processed to 16 non-structural proteins (nsps) by viral proteases (Fang et al. 2012; Rascón-Castelo et al. 2015). The expression of ORF1b involved a –1 ribosomal frameshift in the overlapping ORF1a/1b region (den Boon et al. 1991). ORF1b is composed of nsp9 (RNA-dependent RNA polymerase), nsp10 (helicase), nsp11 (endonuclease) and nsp12 (unknown function) (Snijder et al. 2013; Shi et al. 2016). In particular, nsp9 and nsp10 are believed to be the center components during viral replication and genomic transcription (van Dinten et al. 2000). Our latest studies indicated that nsp9 and nsp10 regions of HP-PRRSV not only affected the replication efficiency of HP-PRRSV in vitro but also contributed to its fatal virulence for piglets (Li et al. 2014).

In this study, we found that HP-PRRSV strain JXwn06 infection could produce a truncated form of nsp10 (termed nsp10a). In contrast to the full-length nsp10, nsp10a is probably short of about 70 amino acids at the N terminus. Next, we identified the residues in nsp10 that affect the generation of nsp10a and analyzed the influence of nsp10a on the growth of PRRSV in vitro.

2. Materials and methods

2.1. Cells, viruses and antibody

Porcine alveolar macrophages (PAMs) were obtained from the lung lavage fluid of specific-pathogen-free (SPF) pigs as previously described (Zhang et al. 2009) and maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum and penicillin/streptomycin. The MARC-145 cell line and the human embryonic kidney HEK-293FT cell line were cultured in Dulbecco’s modified Eagle’s medium (DMEM) with 10% FBS at 37°C. Two PRRSV strains, JXwn06 and HB-1/3.9 were isolated and kept in our laboratory (Zhou et al. 2009). Five vaccine strains, HuN4-F112 (GenBank no. unavailable), TJM-F92 (GenBank no. unavailable), JXA1-R (GenBank no. unavailable), RespPRRS MLV (GenBank no. AF066183) and CH-1R (GenBank no. EU807840), were purchased from market. Mouse anti-PRRSV nsp10 monoclonal antibody (mAb) was prepared in our laboratory (Zhang et al. 2017).

2.2. Plasmid construction and DNA transfection

Plasmids were constructed by standard recombinant DNA procedures. A series of eukaryotic plasmids expressing different fragments of nsp10 were constructed. The DNA fragments of nsp10 gene were amplified by PCR using the plasmid pWSK-CMV-JXwn06 as the template with different primer sets (Table 1) and then subcloned into the eukaryotic vector pEGFP-N1 via BamHI/KpnI sites. A stop codon was placed just downstream of inserted DNA fragments to prevent fusing with GFP tag. In this way, the expressed nsp10 fragments will reflect their real sizes as they don’t include any irrelevant amino acid.

Transfection was conducted using Lipofectamine® LTX with Plus™ Reagent (Invitrogen, USA) according to the

Table 1 The primers for construction of nsp10 deletion mutants

| Primer names | Sequences (5´→3´) | Length of PCR products (bp) |
|--------------|-------------------|---------------------------|
| nsp10-F      | GGTACCATGGGGAAGAAGTCCAGAATGTG | 1323 |
| nsp10-R      | GGATCCTCATTCCAGGTCGTGCCAAATAG | 1173 |
| Del50-F      | GGTACCATGGTACAGTGTAAGTGGACAACCCCTTCTAG | 1143 |
| Del50-R      | GGATCCTCATTCCAGGTCGTGCCAAATAG | 1113 |
| Del60-F      | GGTACCATGACAAGGCTCTAGTAGGAGGTG | 1053 |
| Del60-R      | GGATCCTCATTCCAGGTCGTGCCAAATAG | 1083 |
| Del70-F      | GGATCCTCATTCCAGGTCGTGCCAAATAG | 1053 |
| Del70-R      | GGATCCTCATTCCAGGTCGTGCCAAATAG | 1083 |
| Del80-F      | GGATCCTCATTCCAGGTCGTGCCAAATAG | 1053 |
| Del80-R      | GGATCCTCATTCCAGGTCGTGCCAAATAG | 1083 |
| Del90-F      | GGATCCTCATTCCAGGTCGTGCCAAATAG | 1083 |
| Del90-R      | GGATCCTCATTCCAGGTCGTGCCAAATAG | 1083 |

1) F and R stand for forward and reverse primers, respectively.
2) The restriction enzyme sites of KpnI and BamHI were underlined.
manufacturer's instruction. Briefly, HEK-293FT cells grown to 80% confluence in 6-well plates were transfected with transfection mix, which contains 2.5 μg of plasmid, 2.5 μL of Plus reagent and 5 μL of Lipofectamine® LTX in Opti-MEM. HEK-293FT cells were cultured for 24 h post-transfection for protein expression.

2.3. Recovery of recombinant viruses from infectious clones

The infectious clone plasmids of JXwn06 and HB-1/3.9, pWSK-CMV-JXwn06 and pWSK-CMV-HB-1/3.9 were constructed and kept in our laboratory. Site-directed mutants of nsp10 gene were generated by PCR-directed mutagenesis and cloned into plasmid pWSK-CMV-JXwn06 or pWSK-CMV-HB-1/3.9 to generate infectious clones containing expected mutants. All the constructed infectious clones were verified by DNA sequencing.

MARC-145 cells grown to 70–80% confluence in six-well plates were transfected with 2.5 μg of infectious cDNA clones using Lipofectamine® LTX with with Plus™ Reagent. The culture supernatants of MARC-145 cells were harvested at 5 d post-transfection when obvious cytopathic effect (CPE) appeared, and the rescued viruses were designated 'passage-1'. The designed mutant bases in the RNAs of third-passage viruses were confirmed by DNA sequencing.

2.4. Silver staining and mass spectrometric (MS) analysis

The cell lysates of PRRSV-infected cells were resolved by electrophoresis on 10% SDS-PAGE gels and stained using a Silver Stain Kit for Mass Spectrometry (Thermo Scientific, Rockford, IL) according to the manufacturer's protocol. Prestained protein ladder was used for estimating the approximate sizes of separated proteins. The protein band corresponding to nsp10a in the gel was excised and subjected to MS analysis as described previously (Du et al. 2016).

2.5. Western blotting analysis

PRRSV-infected cells were lysed with RIPA buffer and then the cell lysates were cleared by centrifuging at 12000 r min⁻¹ for 20 min. The protein concentrations in the supernatants were quantified by using a bicinchoninic acid (BCA) protein assay kit (Pierce, USA). Every protein samples with 30 μg were first fractionated by SDS-PAGE, and then transferred onto a polyvinylidene fluoride (PVDF) membrane. After blocking with 5% skim milk powder in PBST (0.05% Tween 20), the membrane was probed with anti-nsp10 mAb. The membrane was washed with 0.05% PBST and incubated with horseradish peroxidase (HRP)-conjugated goat antimouse IgG (ZSGB-BIO, China). At last, the membrane was washed and developed with the ECL Western Blotting System (Pierce, USA).

2.6. Immunofluorescence microscopy

MARC-145 cells grown on coverslips in six-well plates were infected with PRRSV strain JXwn06 at multiplicity of infection (MOI) of 0.1. At 12, 16, 20, 24 and 32 h post-infection (hpi), the cells were fixed with 3.7% paraformaldehyde for 10 min at room temperature (RT), and permeabilized with PBS containing 0.1% Triton X-100 and 2% BSA for 10 min, then blocked with 2% BSA in PBS for 30 min. MARC-145 cells were stained with nsp10-specific mAb in a humidity chamber for 1 h at RT. After three times of 5 min washing with PBS, the cells were then incubated with Alexa Fluor 488-conjugated goat anti-mouse IgG F(ab')₂ fragment (Molecular Probes) antibody for 1 h at RT. Nuclear DNA was stained with 4’,6-diamidino-2-phenylindole (DAPI) (Molecular Probes) for 5 min at RT. The images were collected with an Olympus confocal microscope (Fluoview1000, Japan).

2.7. Virus infection and titration

MARC-145 cells grown to full confluence in 24-well plates were infected with RvJX-E69G and RvHB-G69E or their parental viruses (RvJXwn06 and RvHB-1/3.9) at MOI of 0.01 in DMEM with 5% FBS. The titers of viruses in the supernatants at different time points were determined using a microtitration infectivity assay and expressed as 50% tissue culture infective dose per mL (TCID₅₀ mL⁻¹) as previously described (Zhou et al. 2009). Each time point was independently repeated three times.

2.8. Statistical analysis

The data were expressed as means±standard deviations (SD). All statistical analyses were performed using GraphPad Prism version 5.0 software. Statistical significance among different groups was determined by two-way ANOVA analysis of variance.

3. Results

3.1. PRRSVs produce a truncated nsp10 protein in a strain-specific manner

At the beginning of this study, two PRRSV strains, HP-PRRSV JXwn06 and LP-PRRSV HB-1/3.9, were chosen to infect MARC-145 cells, respectively. At 48 hpi, the cell
lysates were prepared and subjected to Western blotting probed by anti-nsp10 mAb. Interestingly, two specific bands, with estimated molecular weight of 50 and 42 kD, were detected in samples from JXwn06 infected MARC-145 cells (Fig. 1-A). The upper band is corresponding to the expected full-length nsp10, while the lower one is probably a truncated form of nsp10. For convenience, we named the full-length one nsp10 and the truncated form nsp10a hereafter. However, unlike JXwn06, HB-1/3.9 infection only produced the full-length nsp10 in MARC-145 cells (Fig. 1-A). Next, we tested the expression of nsp10 products in PRRSV-infected PAMs, the target cells for PRRSV. The result in PAMs is similar to that in MARC-145 cells, indicating the production of nsp10a is not dependent on specific cell types (Fig. 1-A).

To determine if the production of nsp10a is a unique phenomenon for the strain JXwn06 or it’s universal for other PRRSV strains, five PRRSV vaccine strains that were easily available from market were chosen to infect MARC-145 cells. The infection of TJM-F92, JXA1-R, RespPRRS MLV or CH-1R produced both nsp10 and nsp10a, while HuN4-F112 infection only generated nsp10 (Fig. 1-B). Taken together, the generation of nsp10a is strain-specific.

3.2. The expression kinetics of nsp10 products during PRRSV infection

In order to investigate the kinetics of nsp10 and nsp10a during PRRSV infection, MARC-145 cells were infected with JXwn06 and the infected cells were subjected to confocal microscopy and Western blotting analysis at indicated time points post-infection using anti-nsp10 mAb. The fluorescence signal appeared at 12 hpi and its intensity increased as the time goes on (Fig. 2-A). By 32 hpi, bright fluorescent foci appeared in ~80% of the cells (Fig. 2-A). The intracellular localization of PRRSV nsp10 is similar to that of PRRSV nsp2, nsp4, nsp7α, nsp7β and nsp8, in the perinuclear region of infected cells (Li et al. 2012). In Western blotting analysis, the full-length nsp10 was first detected at 24 hpi, reached peak level at 60 hpi and started to wane at 72 hpi in JXwn06-infected cells (Fig. 2-B). While nsp10a appeared later than 24 hpi, however, increased until 72 hpi and became the predominant form of nsp10 products at the later time points (Fig. 2-B).

Fig. 1 The production of nsp10a is in a strain-specific manner. A, identification of nsp10 products in JXwn06- or HB-1/3.9-infected cells. MARC-145 cells or PAMs were infected with JXwn06 and HB-1/3.9 at MOI=0.1. At 48 h post-infection (hpi), the cells were harvested and the cell lysates were separated by SDS-PAGE followed by Western blotting with the nsp10-specific mAb. B, Western blotting of nsp10 products in MARC-145 cells infected with different vaccine strains.

Fig. 2 The expression kinetics of nsp10 products in porcine reproductive and respiratory syndrome virus (PRRSV)-infected MARC-145 cells. A, identification of nsp10 products by immunofluorescent assay (IFA). MARC-145 cells were infected with JXwn06 at multiplicity of infection (MOI) 0.1. The infected cells were subjected to IFA using anti-nsp10 mAb at indicated time points post-infection. B, the expression trend of nsp10 products in JXwn06-infected cells. MARC-145 cells were infected with JXwn06 at MOI of 0.01. The cell lysates were separated by SDS-PAGE and subjected to Western blotting with anti-nsp10 mAb. hpi, hours post-infection. Bar=10 µm.
3.3. Nsp10a may be an N-terminally truncated form of full-length nsp10

Since nsp10a could be recognized by anti-nsp10 mAb, nsp10a is likely a truncated form of nsp10. To confirm the identity of nsp10a, JXwn06-infected cell lysates were harvested at 48 hpi and separated by SDS-PAGE, and then the gels were stained using a Silver Stain Kit (Thermo, Rockford, IL). The nsp10a bands in gels were identified by comparing to that of the protein standards as well as the full-length nsp10. The nsp10a-containing bands were excised from the gels and subjected to mass spectrometry (MS) analysis. The identified peptide sequences were searched on the NCBI website using the protein BLAST (blastp) suite. The peptides that matched to PRRSV nsp10 were aligned with the amino acid sequences of nsp10 and showed in Fig. 3-A. In Fig. 3-A, the green lines represent the identified peptides whose amino acid sequences are identical to that shown above the green lines, while the number of green lines stand for how many times of the peptides been identified in separate experiments. The results of MS revealed that nsp10a was indeed a truncated form of nsp10. The lengths of the peptides mostly ranged from 9 to 20 amino acids except one with 34 amino acids and all the identified peptides mapped to the sequence of nsp10 between amino acids 78 and 423 (Fig. 3-A).

To identify the size of nsp10a, a series of nsp10 proteins with designed deletions were expressed in vitro and compared to nsp10a from virus-infected cell lysates. Five recombinant plasmids based on the eukaryotic vector pEGFP-N1 were constructed by deletion mutagenesis, which expressed nsp10 proteins with deletions ranging from 50 to 90 amino acids at the N-terminus. HEK-293FT cells transfected with the constructed plasmids were harvested 24 h post-transfection, and subjected to Western blotting analysis by anti-nsp10 mAb. The results indicated that the eukaryotic expressed full-length nsp10 protein had a gel migration pattern similar to that of natural nsp10 protein from JXwn06-infected cells (Fig. 3-B). While the truncated nsp10 with 70 amino acids deletion migrated as fast as nsp10a in gel. Western blotting results coupled with the MS data revealed that nsp10 and nsp10a may share the same C-terminus and nsp10a is probably short of about 70 amino acids in the N-terminus.

3.4. Amino acid 69-Glu in nsp10 is the key residue that affects the production of nsp10a

As shown above, the production of nsp10a is strain-dependent. However, the determinants affecting the generation of nsp10a are still unknown. To address this question, four chimeric viruses, RvHJn10, RvHJn9n10, RvJHn10, and RvJHn9n10, were used to infect MARC-145 cells. The four chimeric viruses were made previously by swapping nsp10 gene alone or nsp9 and nsp10 genes together between JXwn06 and HB-1/3.9 (Li et al. 2014). The infected cell lysates were prepared at 48 hpi for Western blotting analysis. The Western blotting results showed that when nsp10 gene or both nsp9 and nsp10 genes in JXwn06 were substituted by that of HB-1/3.9, the chimeric viruses RvJHn10 and RvJHn9n10, which replaced nsp10 gene or nsp9 and nsp10 genes on HB-1/3.9 backbone with that of JXwn06, produced nsp10a (Fig. 4-A). In contrast, the chimeric viruses RvHJn10 and RvHJn9n10, which replaced nsp10 gene or nsp9 and nsp10 genes on HB-1/3.9 backbone with that of JXwn06, produced nsp10a (Fig. 4-A). These data suggested that the generation of nsp10a was dictated by nsp10 gene itself, but not other genes of PRRSV.

Sequence alignment of nsp10 between JXwn06 and HB-1/3.9 showed that there were five different amino acid residues, which were located at 11, 51, 69, 296, 408 amino acids in nsp10 (Fig. 4-B). To determine the key determinants that involved in nsp10a production, we constructed five virus on the backbone of JXwn06 by mutating the five
amino acids one by one to the corresponding residues of HB-1/3.9. Western blotting analysis showed that the rescued viruses RvJX-G11M, RvJX-E69G and RvJX-M296V could not generate nsp10a in infected cells. The results suggested that the amino acids at 11, 69 and 296 affect the production of nsp10a (Fig. 4-C). Then, we rescued three viruses on the backbone of HB-1/3.9 by mutating M to G, G to E and V to M at 11, 69 and 296 in nsp10, respectively. Further analysis revealed that only the rescued virus RvHB-G69E partially gained the ability to produce nsp10a (Fig. 4-D). Together, the data indicated that the amino acid 69-Glu in nsp10 is the key amino acid determining the production of nsp10a.

3.5. Nsp10a is not essential for PRRSV replication in cell culture

To analyze the effect of the production of nsp10a on PRRSV replication, the growth kinetics of RvJX-E69G and RvHB-G69E were evaluated in MARC-145 cells. The results showed that RvJX-E69G and RvHB-G69E exhibited comparable growth kinetics to their parental viruses at indicated time points (Fig. 5). As shown above, RvJX-E69G infection could not produce nsp10a while RvHB-G69E infection could generate nsp10a, which were both different from their parental viruses, respectively. Therefore, the generation of nsp10a had little effect on PRRSV replication in vitro.

4. Discussion

The truncated form of nsp10 protein (nsp10a) generated in PRRSV-infected cell lysates was reported for the first time in the present study. We have demonstrated that the generation of nsp10a is dictated by nsp10 gene, but we cannot rule out the possibility that viral other genes are involved in this process. In fact, as shown in Fig. 4-A, nsp9 and nsp10 genes together displayed a synergistic effect for nsp10a production. Although the overall quantities of nsp10 products in RvHJn9n10 group are less than that in RvHJn10 group, the amount of nsp10a in RvHJn9n10 group is more than that in RvHJn10 group (Fig. 4-A). Therefore, other genes of PRRSV, such as nsp9, although did not determine the production of nsp10a, but might influence the efficiency of nsp10a production.

Previous study has shown that nsp9 and nsp10 of HP-PRRSV contributed to its fatal virulence (Li et al. 2014). In the present study, we demonstrated that the production of nsp10a had little influence on PRRSV replication. Whether nsp10a correlated with viral virulence is unknown. Based on the results of this study, we can infer that nsp10a was probably not correlated with PRRSV virulence, as both the
CHsx1401, which has an Arg at 69th amino acid in nsp10 and Table 2). Another example is the NADC30-like strain and it could not produce nsp10a in infected cells (Fig. 1-B). The HuN4-F112 strain has a Gly at the site of 69 in nsp10, generated nsp10a upon infection (Fig. 1-B). However, amino acid 69-Glu in nsp10 (Table 2) and these four strains shown in Fig. 1-B. Four out of five vaccine strains have production of nsp10a, can be further verified by the result of the amino acid 69-Glu in nsp10 is the key residue determining the production of nsp10a. Since the anti-nsp10 mAb generated in our laboratory could not recognize the European PRRSV strains, we could not determine whether European PRRSV strains can generate nsp10a, such as European strain GZ11-G1 (Zhang et al. 2017).

The mechanism to generate different forms of PRRSV nsp10 remains unclear. Various mechanisms are employed to generate different forms of proteins, such as leaky scanning, alternative splicing, proteolytic cleavage and translation reinitiation (Liu et al. 1991a, b; Carter and Roizman 1996; Firth et al. 2011). For example, a novel structural protein in PRRSV termed ORF5a was generated via leaky ribosomal scanning (Firth et al. 2011). ORF5a is encoded by an alternative ORF which is not in-frame with that of GP5, although they are expressed from the same subgenomic mRNA (sgRNA) (Johnson et al. 2011). Thus, the amino acid sequences of ORF5a and GP5 have no homology. Another example is the newfound accessory protein NS7a encoded by porcine deltacoronavirus (PDCoV) (Fang et al. 2017). NS7a is encoded by a separate sgRNA with a non-canonical transcription regulatory sequence (TRS). The separate sgRNA encoding NS7a is identical to the 3′ end of the sgRNA encoding NS7, which is generated by splicing the TRS and coding region of NS7. The earlier study showed that PRRSV nsp2 existed as several isoforms with apparently different C termini in virus-infected MARC-145 cells. Those different nsp2 isoforms were likely processed under proteolytic cleavage occurred cotranslationally or via −1 and −2 ribosomal frameshifting (Han et al. 2010; Fang et al. 2012).

Obviously, nsp10a was not derived from alternative splicing or translation reinitiation as the former does not occur, and the latter would mean an M at the N-terminus. There are two possible explanations for nsp10a production, proteolytic cleavage or degradation of nsp10. According to the amount of nsp10a vs. that of the full-length nsp10 at different time points post-infection, it seemed that the increase of nsp10a was negatively correlated with the decrease of nsp10 (Fig. 2-B). We propose that nsp10a may be generated through the proteolytic cleavage of the full-length nsp10. To test if the viral proteases, like nsp4, nsp2, nsp1α and nsp1β, could cleave nsp10, they were co-expressed with JXwn06 nsp10 alone or in combination in HEK-293FT cells. However, the Western blotting results showed that all these viral proteases could not cleave nsp10 (data not shown). We also established a stable MARC-145 cell line that expresses a double tagged nsp10, HA-nsp10-Flag. The recombinant protein HA-nsp10-Flag

Fig. 5 Comparison of the growth kinetics of RvJX-E69G and RvHB-G69E with their parental viruses. MARC-145 cells were infected RvJX-E69G, RvHB-G69E, RvJXwn06 and RvHB-1/3.9 at multiplicity of infection (MOI) of 0.01, respectively. Virus titers at 12 to 72 h post-infection (hpi) were determined by microtitration infectivity assays. The data were shown as means±standard deviations (error bars) from three independent trials.
was not cleaved whether the nsp10-expressing cell line was infected with JXwn06 or not (data not shown).

Equine arteritis virus (EAV) is the prototype of the Arterivirus genus and the studies of EAV provided a general model for PRRSV research. The crystal structure of PRRSV nsp10 is unavailable, whereas the crystal structure of EAV nsp10 has been described recently (Deng et al. 2014). EAV nsp10 contained an N-terminus zinc-binding domain (ZBD), a superfamily 1 helicase domain (HEL1) in the C-terminal half and a linker connecting the two domains, and ZBD interacts extensively with the HEL1 domain. The integrity of nsp10 is essential for its helicase properties (Deng et al. 2014) and this was in line with the previous studies of Arterivirus nsp10, which showed that the ZBD was required for its helicase activity and involved in genome replication (Seybert et al. 2000, 2005). PRRSV nsp10 has ATPase activity and can unwind dsRNA and dsDNA in a 5’ to 3’ direction (Bautista et al. 2002; Zhang et al. 2015).

Protein homology modeling revealed that the structural characteristics of PRRSV nsp10 were similar to that of EAV nsp10. In EAV, the N-terminus 62 amino acids of nsp10 is the ZBD (Bautista et al. 2002). Accordingly, the N-terminus 64 amino acids in PRRSV nsp10 is the ZBD (http://www.uniprot.org/uniprot/I6XH19). Nsp10a was a truncation of nsp10. The structural deficiency of nsp10a would affect or even block helicase activity. Thus, we inferred that the generation of nsp10a might be unfavorable for PRRSV replication. However, the results showed that the production of nsp10a exerted little influence on the replication of PRRSV in cell culture. It is possible that inactivation of helicase function is instead favorable at later stages of infection, when active RNA replication should be replaced by active packaging and virus egress. Since the tests in the present study were conducted for only 72 hpi, experiments with longer duration are needed to further confirm the speculation both in vitro and in vivo.

5. Conclusion

In summary, a truncated form of nsp10 termed nsp10a is found in PRRSV-infected cell lysates. Nsp10a may share the same C-terminus with nsp10 but differ in their N-termini. The Glu-69 in nsp10 is the key amino acid determining nsp10a production and the generation of nsp10a imposed little effect on PRRSV replication in cell culture.

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