36

SUBGENOMIC RNA7 IS TRANSCRIBED WITH DIFFERENT LEADER-BODY JUNCTION SITES IN PRRSV (STRAIN VR2332) INFECTION OF CL2621 CELLS

Kay S. Faaberg, Margaret R. Elam, Chris J. Nelsen, and Michael P. Murtaugh

Department of Veterinary PathoBiology
University of Minnesota
1971 Commonwealth Avenue
St. Paul, Minnesota 55108

1. ABSTRACT

Porcine reproductive and respiratory syndrome virus (PRRSV), like all members of the order Nidoviridae, is expressed in the infected cell as a nested set of subgenomic (sg) RNAs with a common 5′-leader sequence. We have determined that the 5′-leader sequence for the US prototype strain (VR2332, Collins, *et al.*, 1992) is distinct from the European prototype strain [Lelystad (LV); Wensvoort, *et al.*, 1991, Meulenberg *et al.*, 1993a], yet these two strains use almost the same sequence for downstream sites of 5′-leader-body junction formation. Analysis of VR2332 genomic sequence identified several potential 5′-leader-body junction sequences upstream of open reading frame (ORF) 7, coding for the nucleocapsid protein, that could be used for generation of VR2332 sgRNA7 transcripts. Sequence determinations of RT-PCR-generated cDNA clones of sgRNA7 identified two species of RNA7 transcripts in infected cells, one utilizing a leader-body junction sequence (AUAACC) 123 nucleotides upstream of the AUG start site and one utilizing a sequence (UAAACC) 9 nucleotides upstream of the AUG start site for ORF7 translation.

2. INTRODUCTION

PRRSV causes severe respiratory problems in young pigs and reproductive failures in infected sows. This viral disease first appeared in North America in 1987 (Keffaber,

Coronaviruses and Arteriviruses, edited by Enjuanes *et al.*
Plenum Press, New York, 1998

1989) and in Europe in 1990 (Paton *et al.*, 1991), and has since spread worldwide (Halbur *et al.*, 1996). The positive sense, single stranded RNA genomes of both a North American strain (VR2332, approximately 15 kilobases (kb) in length, Collins, *et al.*, 1992) and a European strain (LV, 15.1 kb in length, Meulenberg *et al.*, 1993b) of PRRSV encode at least 8 ORFs. Both PRRSV genomes are transcribed in the infected cell into a nested set of 7 RNAs with a common 5′-leader sequence. Each RNA transcript, except sgRNA7, encodes more than one ORF, but is believed to only express the 5′ terminal ORF sequence. Protein sequence comparison of ORFs 2–7 of these two viruses reveal that VR2332 and LV are markedly different, however. Amino acid homologies for the ORFs range from 59% (ORF 5) to 70% (ORF 6) identity (Murtaugh, *et al.*, 1995).

We report that these two strains of PRRSV code for 5′-leader sequences of only moderate nucleotide similarity and that two leader-junction sites are utilized to produce sgRNA7 transcripts for strain VR2332. Previously, only one leader-junction site for sgRNA 7 had been identified for the LV strain (Meulenberg, *et al.*, 1993a) and for a strain isolated in Japan (EDRD-1), which is more closely related to a North American strain (VR2385) than LV in the region encoding ORFs 5–7 (Saito, *et al.*, 1996).

3. MATERIALS AND METHODS

A genomic library of the VR2332 strain of PRRSV (Collins, *et al.*, 1992) produced cDNA clones representing partial VR2332 sgRNAs with VR2332 5′-leader sequence (data not shown). Forward primer 658P1/ (CAGGAGCTGTGACCATTGGCA) was constructed from a leader sequence clone (c658). 658P1/ and 3′ RACE primers (Qt, CCAGTGAGCAGAGTGACGAGGACTCGAGCTCAAGCTTTTTTTTTTTTTTTT, and Qi, GAGGACTCGAGCTCAAGC) were used to amplify species of sgRNA7 from VR2332 infected CL2621 cell total RNA using standard RT-PCR techniques. The total RNA from these infected cells (RNAeasy, Qaigen Inc., Santa Clarita, CA) was reversed transcribed to obtain almost full-length cDNAs representing sgRNA7. The cDNA was amplified by PCR and the RT-PCR (GeneAmp RNA PCR, Perkin Elmer, Foster City, CA) DNA products were analyzed by gel electrophoresis (data not shown). The DNA products of the approximate size were TA-cloned and sequenced. Taq DyeDeoxy™ Terminator Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, CA) was utilized to generate the sequence of 10 clones of VR2332 sgRNA7. Resulting sequences were analyzed and aligned using the ASSEMBLE and LINEUP programs in the software package by the Genetics Computer Group (GCG), University of Wisconsin, USA.

4. RESULTS

4.1. VR2332 5′-Leader Sequence

The 5′-leader sequence determined to date for strain VR2332 is 170 nucleotides in length and will be published elsewhere (manuscript in preparation). Primer extension analysis has demonstrated that 20 nucleotides at the 5′-end of the sequence remain uncloned (data not shown). Accounting for the missing sequence, we predict that the VR2332 5′-leader contains approximately 190 nucleotides. The 5′-leader sequence of the LV strain is 221 nucleotides in length (Meulenberg, *et al.*, 1993a; personal communication), which is about 31 nucleotides longer than the VR2332 leader sequence. Sequence

comparison indicates that PRRSV 5′-leader sequence for strain VR2332 (incomplete) and strain LV leader sequence exhibit 81% sequence similarity [Needleman-Wunsch comparison (Gap creation penalty:3, Gap extension penalty:0.1)].

4.2. VR2332 sgRNA7 Leader-Body Junction Sequences

Computer analysis of strain VR2332 identified 4 possible leader-body junction sites located downstream of the initiation AUG codon for ORF6 and upstream of the ORF7 AUG codon. These sites were located at nucleotides 2549–2554 (UUCACC), 2772–2777 (AUAACC), 2871–2876 (UUAAAC), and 2886–2891 (UAAACC) of the submitted VR2332 sequence (GENBANK submission U00153). Sequence analysis of 10 DNA clones of sgRNA7 indicated that at least two leader-body junction sites were utilized in the production of VR2332 ORF 7 transcripts in CL2621 cells. The most abundant 5′-leader-body junction site, representing 7 clones, identified the AUAACC sequence 123 nucleotides proceeding the starting AUG of ORF7 (nucleotides 2901–2903). Another sequence (UAAACC), located 9 nucleotides proceeding the ORF7 AUG (3 clones), was also used for sgRNA7 transcription. Each set of similar clones displayed identical leader-body junction sequences.

4.3. PRRSV Strain Comparison of Utilized 5′-Leader-Body Junction Sequences

A comparison was made between two other PRRSV strains for which leader-body junction sequences have been determined. Two clones of sgRNA7 for a Japanese strain, EDRD-1, have been analyzed. ERDR-1 was determined to be 94.3% similar in ORF7 to strain VR2385 (Saito, *et al.*, 1996), a North American strain. The investigators found that only the sequence (AUAACC), 123 nucleotides between the end of the leader-body junction sequence and the iniation codon for ORF7, was used for sgRNA7 transcription. These results directly correspond with the major sgRNA7 leader-body junction sequence (AUAACC) for strain VR2332. Six clones derived from sgRNA7 for LV, a European strain, have been reported. All of the LV clones possessed a leader-body junction site (UUAACC), spanning nucleotides 14573–14578 (GENBANK accession number M96262), which is located 9 nucleotides upstream of the ORF7 initiating AUG. This sequence varies by one nucleotide from the second sequence (UAAACC) used in VR2332 sgRNA7 transcription., and corresponds to the same number of nucleotides between the junction sequence and the start site for translation of ORF7.

5. DISCUSSION

All arteriviruses have been shown to utilize similar leader-body junction sequences [Saito, *et al.*, 1996 (EDRD-1); Meulenberg, *et al.*, 1993a (LV); Chen *et al.*, 1993 (lactate dehydrogenase-elevating virus(LDV)); Zeng, *et al.*, 1995 (simian hemorrhagic fever virus (SHFV)); den Boon, *et al.*, 1996 (equine arteritis virus (EAV))]. The consensus sequence (U/A)(U/A)AACC is used in transcription of sgRNA7 for all strains of PRRSV, and minor variations on this basic hexanucleotide sequence is used in production of all PRRSV sgRNAs. However, although the 5′-leader-junction sequences are similar, other regions of the PRRSV genome are dissimilar in sequence. The 3′-end of the genomes of VR2332 and LV are quite distinct (Murtaugh, *et al.*, 1995) and as related in this abstract, the North

American prototype (VR2332) and the European prototype (LV) display dissimalarity in both the length and the sequence of their 5′-leader regions. In addition, sequences upstream of the reported structural regions show considerable genotypic divergence (manuscript in preparation).

Two leader-junction motifs were shown to be utilized in VR2332 sgRNA7 (encoding the nucleocapsid (N) protein) in infected CL2621 cells. No other arterivirus, including PRRSV strains EDRD-1 and LV, has been shown to exhibit a variation in sgRNA expression for a critical structural protein, although EAV can express multiple forms of sgRNA3 (den Boon, *et al.*, 1996). Our evidence suggests that the predominant VR2332 sgRNA7 utilizes the site 123 bp upstream of ORF7, and the second site is used less frequently. We are presently determining whether infection of porcine alveolar macrophages, the natural host cell for PRRSV infection, will produce similar results and if other PRRSV sgRNA7 5′-leader-body junctions sites may be utilized at a low frequency. LV sgRNA7 appears to utilize the sequence 9 bp upstream of LV ORF 7 exclusively (Meulenberg, *et al.*, 1993a). It will be interesting to explore the functional sugnificance of the differences in 5′-leader-body junction sequences utilized by the North American and European strains of PRRSV.

ACKNOWLEDGMENTS

The authors wish to thank Judy Laber and Dan Strom of the University of Minnesota Advance Genetic Analysis Center for sequencing expertise and Thy M. Truong and Sara Proman for technical assistance. Boehringer Ingelheim Animal Health, Inc. provided financial support for this project.

REFERENCES

Chen, Z., Kuo, L., Rowland, R.R.R., Even, C., Faaberg, K.S., and Plagemann, P.G.W., 1993, Sequences of 3′ end of genome and of 5′ end of open reading frame 1a of lactate dehydrogenase-elevating virus and common junction motifs between 5′ leader and bodies of seven subgenomic mRNAs, *J. Gen. Virol.* **74**: 643–660.

Collins, J.E., Benfield, D.A., Christianson, W.T., Harris, L., Hennings, J.C., Shaw, D.P., Goyal, S.M., McCullough, S., Morrison, R.B., Joo, H.S., Gorcyca, D., and Chladek, D., 1992, Isolation of swine infertility and respiratory syndrome virus (isolate ATCC VR-2332) in North America and experimental reproduction of the disease in gnotobiotic pigs, *J. Vet. Diagn. Invest.* **4**: 117–126.

den Boon, J.A., Kleijnen, M.F., Spaan, W.J.M., and Snijder, E.J., 1996, Equine arteritis virus subgenomic mRNA synthesis: Analysis of leader-body junctions and replicative-form RNAs, *J. Virol.* **70**: 4291–4298.

Halbur, P.G., Paul, P.S., Meng, X.J., Lum, M.A., Andrews, J.J., and Rathje, J.A., 1996, Comparative pathogenicity of nine US porcine reproductive and respiratory syndrome virus (PRRSV) isolates in a five-week-old cesarean-derived, colostrum-deprived pig model, *J. Vet. Diagn. Invest.* **8**: 1120.

Keffaber, K.K., 1989, Reproductive failure of unknown etiology, *Am. Assoc. Swine Pract. Newsl.* **1**: 1–9.

Meulenberg, J.J., de Meijer, E.J., and Moormann, R.J., 1993a, Subgenomic RNAs of Lelystad virus contain a conserved leader-body junction sequence, *J. Gen. Virol.* **74**: 1697–1701.

Meulenberg, J.J., Hulst, M.M., de Meijer, E.J., Moonen, P.L., den Besten, A., de Kluyver, E.P., Wensvoort, G., Moormann, R.J., 1993b, Lelystad virus, the causative agent of porcine epidemic abortion and respiratory syndrome (PEARS), is related to LDV and EAV, *Virology* **192**: 62–72.

Murtaugh, M.P., Elam, M.E., and Kakach, L.T., 1995, Comparison of the structural protein coding sequences of the VR-2332 and Lelystad virus strains of the PRRS virus, *Arch. Virol.* **140**: 1451–1460.

Paton, D. J., Brown, I.H., Edwards, S., and Wensvoort, G., 1991, Blue ear disease of pigs, *Vet. Record* **128**: 617.

Saito, A., Kanno, T., Murakami, Y., Muramatsu, M., Yamaguchi, S., 1996, Characteristics of major structural protein coding gene and leader-body sequence in subgenomic mRNA of porcine reproductive and respiratory syndrome virus isolated in Japan, *J. Vet. Med. Sci.* **58**: 377–80.

Wensvoort, G., Terpstra, C., Pol, J.M.A., ter Laak, E.A., Bloemraad, M., de Kluyver, E.P., Kragten, C., Van Buiten, L., den Besten, A., Wagenaar, F., Broekhuijsen, J.M., Moonen, P.L.J.M., Zetstra,T., de Boer, E.A., Tibben, H.J., de Jong, M.F., van't Veld, P., Groenland, G.J.R., van Gennep, J.A., Voets, M.T., Verheijden, J.H.M., and Braamskamp, J., 1991, Mystery swine disease in the Netherlands: the isolation of Lelystad virus, *Vet. Quarterly* **13**: 121–130.

Zeng, L., Godeny, E.K., Methven, S.L., and Brinton, M.A., 1995, Analysis of simian hemorrhagic fever virus (SHFV) subgenomic RNAs, junction sequences, and 5′ leader, *Virology* **207**: 543–548.