Nanopore-Based Direct RNA-Sequencing Reveals a High-Resolution Transcriptional Landscape of Porcine Reproductive and Respiratory Syndrome Virus

ABSTRACT

The TRS-mediated discontinuous transcription process is a hallmark of Arteriviruses. Precise assessment of the intricate sub genomic RNA (sg mRNA) populations is required to understand the kinetics of viral transcription. It is difficult to reconstruct and comprehensively quantify splicing events using short-read sequencing, making the identification of transcription-regulatory sequences (TRS) particularly problematic. Here, we applied long-read direct RNA sequencing to characterize the recombined RNA molecules produced in porcine alveolar macrophages during early passage infection of porcine reproductive and respiratory syndrome virus (PRRSV). Based on sequencing two PRRSV isolates, namely XM-2020 and GD, we revealed a high-resolution and diverse transcriptional landscape in PRRSV. The data revealed intriguing differences in sub genomic recombination types between the two PRRSVs while also demonstrating TRS-independent heterogeneous subpopulation not previously observed in Arteriviruses. We find that TRS usage is a regulated process and share the common preferred TRS in both strains. This study also identified a substantial number of TRSmediated transcript variants, including alternative-sg mRNAs encoding the same annotated ORF, as well as putative sg mRNAs encoded nested internal ORFs, implying that the genetic information encoded in PRRSV may be more intensively expressed. Epigenetic modifications have emerged as an essential regulatory layer in gene expression. Here, we gained a deeper understanding of m5C modification in poly(A) RNA, elucidating a potential link between methylation and transcriptional regulation. Collectively, our findings provided meaningful insights for redefining the transcriptome complexity of PRRSV. This will assist in filling the research gaps and developing strategies for better control of the PRRS.