Research Project: Swine Viral Diseases Pathogenesis and Immunology

Location: Virus and Prion Diseases of Livestock

Title: *The rolling-circle melting-pot model for porcine circovirus DNA replication*

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Technical Abstract: A stem-loop structure, formed by a pair of inverted repeats during DNA replication, is a conserved feature at the origin of DNA replication (Ori) among plant and animal viruses, bacteriophages and plasmids that replicate their genomes via the rolling-circle replication (RCR) mechanism. Porcine circovirus (PCV) replicates its single-stranded DNA genome by the RCR mechanism via a double-stranded intermediate according to the "melting-pot" model instead of the "cruciform structure" model. In the melting-pot model, the replication proteins (Rep) bind the cognate nucleotide sequence at the Ori and destabilize the palindromic sequences, but there is no formation of a cruciform structure. Instead, the Rep protein-complex induces a sphere of instability -- the melting-pot. Within this destabilized environment, all four strands of the inverted repeats are in a "melted" state. There is a lack of hydrogen bonding between the plus- and minus-strand genomes to maintain any stable double-helix conformation, but the four inverted-repeat strands remain in close proximity to each other and are juxtaposed in a four-stranded tertiary structure. The destabilized "melting-pot" environment at the Ori, with all four strands of the palindrome sequences in the "melted" state and the availability of two templates simultaneously during initiation and termination of DNA replication, contribute to the flexibility as well as the increased mutation frequency at the Ori. Evidence of template-strand switching, in which both the minus-genome and the corresponding palindromic sequence may served as templates, was provided by nucleotide sequence analyses. The results prominently display the "inverted repeats correction/conversion" mechanism inherent in the melting-pot replication model. Previous studies on bacterial plasmids, geminivirus and parvovirus indicate that a palindrome at the Ori of each respective system is essential for RCR DNA replication. However, the involvement of the palindrome with respect to the initiation or termination of DNA synthesis was not conclusive. Mutagenesis studies with monomeric PCV genomes showed that transfection with viral constructs containing mis-matched palindromic sequences incapable of forming a stem-loop structure yielded progeny viruses with complementary palindromic sequences. However, these studies were not able to distinguish whether the mis-matched stem-loop genomes were synthesized or that only genomes containing the repaired and matched stem-loop structure were preferentially packaged into infectious virions. A head-to-tail tandem construct of PCV capable of generating unit-length genomic DNA in Escherichia coli was

employed to examine the role of the stem-loop structure with respect to the RCR initiation and termination process. The advantage of using a head-to-tail tandem construct is that the initiation and termination sites for generation of the unit-length viral genomes are physically separated, which allows independent examination of the initiation and the termination processes. Nucleotide substitution mutational analysis showed that a pair of inverted repeats capable of forming a stem-loop structure was essential for termination, but not for initiation of DNA replication. The results also demonstrated that it is the stem-loop configuration, not nucleotide sequence specificity, which is critical for terminating RCR DNA replication. Examples of template-strand switching, palindrome regeneration, inverted repeats correction and conversion during PCV DNA replication will be discussed.

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