6. SUMMARY

DNA cleavage and ligation are pivotal steps for initiation and termination of genome replication via the rolling circle mechanism. After recruitment of the replicon-encoded initiator protein, replication is initiated by introduction of a strand- and site-specific nick within the double-stranded origin of replication (*dso*). The resulting free 3'-hydroxyl group serves as a primer for DNA synthesis, while the 5'-phosphate group is displaced during DNA replication. After at least one round of replication, the nascent strand is cleaved again within the regenerated origin and the 5'-phosphate end is ligated to the newly created 3'-hydroxyl group resulting in release of unit-length monomers. Due to characteristic sequences found within the origin of replication and the replication proteins, genome amplification of porcine circoviruses type 1 and type 2 (PCV1 and PCV2) is assumed to be mediated by rolling circle replication.

This study demonstrated for the first time the ability of Rep and Rep' of PCV to introduce and reseal strand discontinuities within the origin of replication *in vitro*. Rep and Rep' cleave the viral strand between nucleotides 7 and 8 within the conserved nonamer 5'-$T^{1}AGTATTAC^{9}$-3' and become covalently attached to the 3'-cleavage product. The conserved nonamer and the adjacent sequences *downstream* and *upstream*, which presumably form a hairpin structure, are necessary for cleavage. Since PCV Rep and Rep' are also capable of resealing the viral single-stranded DNA, participation of these proteins in termination of replication is suggested. In this context, joining was strictly dependent on preceding substrate cleavage as well as close proximity of origin fragments presumably accomplished by base pairing.

Cleavage of origin fragments *in vitro* depends on conserved motives I, II and III. Mutation analysis identified tyrosine-93 as the catalytic amino acid. In contrast, the GKS box is not essential for DNA cleavage, although PCV Rep was proven to hydrolyse ATP *in vitro*. Formation of homo- and heterocomplexes of the replication proteins was observed. This supports the hypothesis that Rep/Rep' form a dimer or multimer for initiating and terminating RCR, thereby providing the second catalytic center essential for termination.

The impact of the motives conserved in the replication proteins as well as in the origin of replication was tested in a cell culture-based replication asay. Integrity of conserved motives I, II, III as well as the GKS box and the ability of of PCV Rep/Rep' to promote replication are linked. Recruitment of Rep and Rep' to the viral origin by binding to hexamers H1/H2 of the minimal binding site was demonstrated to be a prerequisite for replication. Cruciform

extrusion for providing the single-stranded DNA conformation of the nonamer indispensable for cleavage and dependency of termination on sequences flanking the nonamer is suggested.