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Porcine circovirus structure and replication: a minireview

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Porcine circoviruses (PCV) are nonenveloped, and have covalently linked circular single stranded (ss) DNA genome. They are the smallest animal viruses capable of independent replication. Circoviruses are thought to use a rolling circle strategy for DNA replication, a unique feature among animal viruses. Three major viral proteins were identified in PCV infected cells: Rep and Rep', involved in genome replication, and the capsid protein. Porcine circoviruses have to rely heavily on the host cell machinery for their replication. Although PCV1 was described in 1974, interest in these viruses has significantly increased only in late nineties, when PCV2 was discovered and linked to several porcine disorders. Our knowledge of porcine circoviruses replication and the basis for their pathogenesis is still very limited. Agricultura 1: 11-14 (2002)

Key words: porcine circovirus; viral proteins; porcine disordes

INTRODUCTION

Porcine circovirus 1 (PCV1) and porcine circovirus 2 (PCV2) belong to the genus Circovirus within the family Circoviridae. Circoviruses may represent a link between animal and plant viruses based on phylogenetic analysis (Mankertz et al. 1997; Meehan et al. 1997), and on proposed recombination between a plant nanovirus and an mammalian RNA virus, probably a calicivirus (Gibbs and Weiller 1999).

Porcine circovirus, today designated as PCV1, was originally isolated as a contaminant from PK-15 cells (Tischer et al. 1974). There is serological evidence that PCV1 is widely distributed in domestic swine both in North America and Europe (Dulac and Afshar 1989, Tischer et al. 1995a). However, no correlation to any porcine disease or disorder was found. On the other hand, PCV2 was found to be a participating agent in several porcine disorders/diseases, such as postweaning multisystemic wasting syndrome (PMWS) (Meehan et al. 1998, Morozov et al. 1998, Kennedy et al. 2000), porcine dermatitis and nephropathy syndrome (Rosell et al. 2000) or congenital tremors type A2 syndrome (Stevenson et al. 2001).

Viral nucleic acid in vivo was detected mainly within the cytoplasm of monocyte/macrophage lineage cells (Rosell et al. 2000) and it appears that immune activation is a key component in the pathogenesis of, at least, PMWS (Krakowka et al. 2001).

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VIRION STRUCTURE

Porcine circoviruses are, with the diameter of viral particle around 17nm, the smallest animal viruses capable of independent replication (Tischer et al. 1982). The nonenveloped virions have icosahedral symmetry (Lukert et al. 1995) and appear to be assembled from single capsid protein. PCV1 capsid protein is larger (36 kDa) (Tischer et al. 1982) than the PCV2 (30 kDa) (Nawagitgul et al. 2000). The virion contains one 1.76 kb molecule of covalently closed circular single-stranded genomic DNA (Tischer et al. 1982). Defective interfering particles have also been described. Their 5S DNA molecule corresponds to a region approximately between nucleotides 340 and 990 with central deletion of variable size left to the origin of replication (Tischer and Buhk 1988, Mankertz et al. 1997).

GENOME STRUCTURE

Virus genomic DNA uses an ambisense coding strategy. Stem-loop with conserved nonamer (5'-TAGTATTAC) in the loop within the 111base pair (bp) origin of replication was detected in the genome. This structure, along with conserved motifs characteristic for proteins initiating the rolling-cycle replication within the coding region, suggests rolling-circle replication of the genome. In addition, sequence CGGCAGCGG/TCAG is found twice in the genome and may represent the binding site for the replication initiator Rep protein. (Mankertz et al. 1997; Meehan et al. 1997, Mankertz et al. 1998a). Nucleotide sequence homology between PCV1 and PCV2 genomes is about 76% (Morozov et al. 1998).

Meehan et al. (1997) used a system for numbering the genome nucleotides according to the convention for geminiviruses: first 'A' residues immediately downstream from the putative nick site in the nonanucleotide is considered to

be nucleotide (nt) number one. According to this system, the origin of replication is located between nucleotides number 1739 and 13. Niagro et al. (1998) started the numbering with the first nucleotide of the nonamer. In Mankertz et al. (1997) the PCV1 origin of replication corresponds to nt 728 - 838. The stem loop is for all groups located between the coding regions of two major open reading frames (ORF) *rep* and C1.

For PCV1 a total of six ORFs, larger than 200 nt (Tischer et al. 1995b, Mankertz et al. 1997), or seven ORFs, capable to encode proteins larger than 5kDa, have been predicted on both DNA strands; three in the encapsidated strand and three or four respectively in the complementary strand of the replicative form (Meehan et al. 1997). Morozov et al. (1998) predicted seven ORFs for PCV1 and six for PCV2 with quite a different distribution of the potential small ORFs. In PCV2 genome they would be all clustered within *rep* ORF, two of them coding in the same direction as *rep*, and two in the opposite direction. As the numbering systems for ORFs differ among the groups, for the purpose of clarity, the ORFs will be described according to the protein they encode.

The largest ORF coded by the encapsidated strand between nt 819 and 1754, represents the *rep* gene (Mankertz et al. 1998b), and encodes two replication initiator proteins, sizes 35.6 kDa (Rep) and 19.2 (Rep') kDa, both essential for PCV replication. Transcription promoter region contains at least one eukaryotic element (TATA box) and the 3'end flanking region of the ORF carries the polyadenylation poly(A) signal (Mankertz and Hillenbrand 2001). The large replication initiator protein (Rep) shares 85% amino acid identity between PCV1 and PCV2 (Morozov et al. 1998).

ORF C1 coding for the capsid protein is located at nucleotide position 1430 to 13 and the transcript is generated from the complementary replicative strand. A transcription regulation region flanking the 5'end of the ORF has, within 258 base fragment (1425 - 1168), a number of eukaryotic transcription regulatory elements: GC-box, CAAT box, and possible rare type of TATA box (TTTCAAAA) within the promoter. The 3'end flanking region carries again a polyadenylation signal at nucleotides 33 - 28 (Mankertz et al. 1998b). This ORF has 65% identical residues between PCV1 and PCV2 (Morozov et al. 1998), yielding a 36 kDa protein for the PCV1 (Tischer et al. 1982), and 30 kDa for the PCV2 (Nawagitgul et al. 2000).

The PCV genome organization with respect to ORFs C1 and *rep* is schematically presented in Fig.1.

VIRUS REPLICATION

Fig. 2 represents an overview of putative steps in PCV replication.

EARLY EVENTS

Mechanism of virus entry into host cells in not known. However it was observed that DEAE-dextran treatment prior to or at the time of infection increased the adsorption and penetration of the virus into cells (Tischer et al. 1987).

The virus replicates in the nucleus, although it also produces intracytoplasmic inclusions in persistently infected cells as observed by electron microscopy (Stevenson et al. 1999). These findings confirm the earlier observations by Tischer et al. (1995b) using an immunofluorescence assay.

PCV genome enters the cell nucleus only by inclusion in the daughter nuclei at the end of mitosis, as it is incapable of penetrating the nucleus membrane on its own (Tischer et al. 1987). The double stranded (ds) replicative form (as supercoiled or relaxed circular molecules), single stranded 13S virion DNA, and small 5S DNA (encapsidated into defective interfering particles) were isolated from extracts of infected cells and from purified virion (Tischer and Buhk 1988).

PCV genome transfection experiments using the DEAE-dextran method or treatment of infected cells with glucosamine indicated that cellular proteins, transiently expressed during the G1 phase of cell cycle, are critical for initiation of viral DNA replication (Tischer et al. 1987).

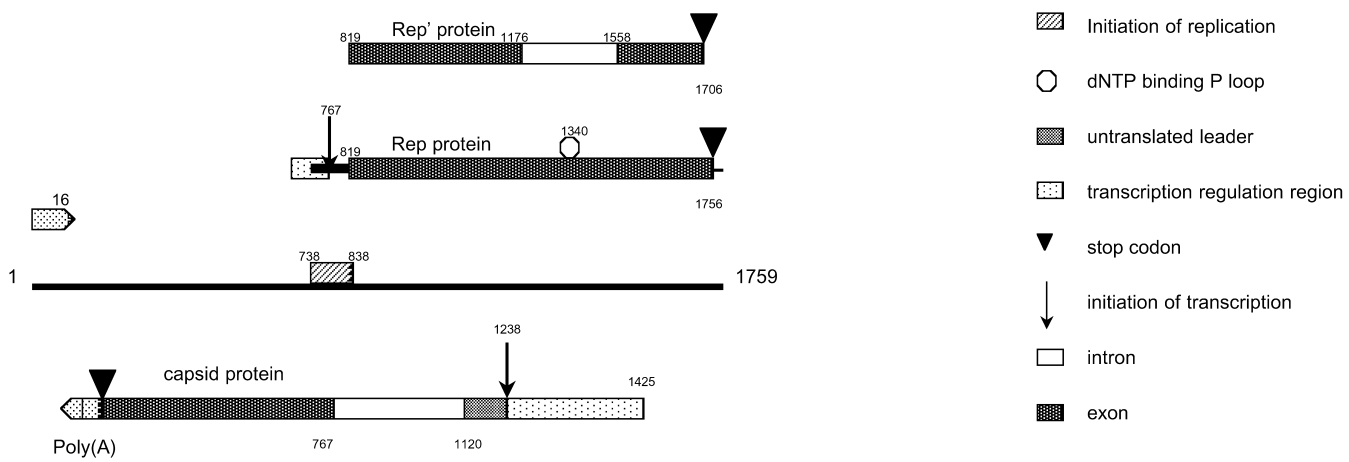

Fig. 1. PCV genome organization with respect to the two major ORFs: rep and C1. The genomic DNA molecule is linearized for the purpose of clarity.

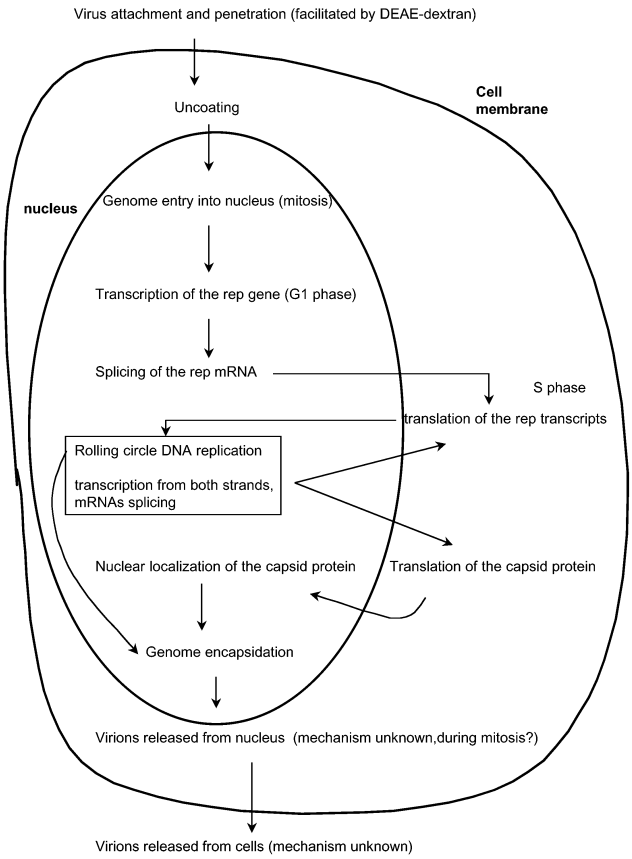

Fig. 2. Putative replication cycle of porcine circoviruses.

REPLICATION, TRANSCRIPTION, TRANSLATION

The hypothesis that replication has to likely start with transcription (and translation) of the *rep* gene by cellular enzymes is supported by the presence of TATA box in the promoter region of the gene (Mankertz and Hillenbrand 2001). Based on the detection of TGAGTCT sequence similar to the consensus binding site for cellular transcription activation factor AP-1 in the PCV1 genome, Tischer et al. (1995b) speculated that AP-1 may be involved in the initial viral transcription. The factor is formed from nuclear protooncogene products c-Jun and c-Fos (and related proteins) expressed during the early G1 cell phase.

The full length *rep* transcript starts at nucleotide 766, passes through nucleotide in position 1, and terminates at poly(A) signal in position 9 – 16 nt. Coding region starts at nucleotide 819, terminates as position 1756 nt, and yields the large Rep protein (35.6 kDa, 312 amino acid residues). The N-terminal region of the Rep protein is similar to the equivalent region in nanovirus Rep, while the C-terminal region is related to an RNA-binding protein of picorna-like viruses (Gibbs and Weiller 1999). Three conserved amino acid motifs in the N-terminus and the dNTP-binding motif carrying P-loop, located in the centre of the Rep protein, are again indicative of rolling cycle replication (Koonin and Ilyina 1993, Mankertz et al. 1997, Markentz et al. 1998a, Meehan et al. 1997).

The *rep* mRNA carries also splice donor and splice acceptor sequences, with an intron between nucleotides 1176 and 1558. Splicing of the *rep* mRNA creates a shorter *rep*' mRNA, in which removal of the intron results in a frame shift and repositioning of the termination codon into position 1706 nt. Translated Rep' protein has only 168 amino acid residues (19.2 kDa), is missing the P-loop region, and the last 48 C-terminal amino acid residues are different from the ones of the Rep protein. Interestingly, the frameshift from Rep to Rep' coincides with the locus at which recombination between nanovirus Reps and the RNA-directed RNA polymerases (2C protein) of caliciviruses was proposed (Gibbs and Weiller 1999, Mankertz and Hillenbrand 2001). However homology studies suggest that porcine circovirus Rep' protein has evolved independently. The Rep protein appears to be corresponding to the early antigen (EA) of PCV, which was thought to be involved in PCV DNA replication (Tischer et al. 1995b).

The two proteins, Rep and Rep' form a functional replication initiator factor critical for synthesis of the ds replicative DNA by rolling circle replication, which also allows for transcription from the complementary strand (Mankertz and Hillenbrand 2001).

The complementary replicative strand serves as a template for the ORF C1 transcript, coding for the capsid protein. The transcription starts at position 1238, the transcript is spliced, joining exon 1 at 1120 nt with exon 2 at position 737 nt, and polyadenylated. The first exon represents an untranslated leader 119nt. Translation starts at the beginning of the second exon and terminates at nt 39. The predicted length of the capsid protein is 233 amino acid residues. PCV2 30 kDa capsid protein does not appear to undergo any co-translational or post-translational modifications, while the 36 kDa capsid protein of PCV1 may undergo post-translation modifications accounting for the difference in molecular mass (Nawagitgul et al. 2000).

LATE EVENTS

The capsid protein has a nuclear localization signal, located within the N-terminal 41 amino acids. Basic amino acid residues at positions 12 to 18 and 34 to 41 are important for the strict nuclear targeting of the capsid protein: 12R-H-R-P-R-S-H18 and 34H-R-Y-R-W-R-R-K41(Liu et al. 2001). The structural protein could be first detected in nucleus 24 hrs post infection classifying it as a late protein; it likely corresponds to the VS antigen described by Tischer et al. (1995b). The capsid proteins can self-assemble into virus like particles (Nawagitgul et al. 2000).

Encapsidation of the genome into viral particles is presumed to occur in the nucleus. A mode of release of the virions from the nucleus and from the cells is not known.

CONCLUSION

As porcine circoviruses must heavily rely on the cell apparatus for their replication, they must inevitably exert some control over functions of cellular proteins and influence cell metabolism by using cellular pathways for their replication.

For example, transient modulation in cell functions and immune cell marker expression has been noted in porcine alveolar macrophages infected with PCV1 (McNeilly et al. 1996).

Shibahara et al. (2000) who confirmed in their work that PCV infected beside other dividing cells also B lymphocytes and directly induced apoptosis in them, noted that this effect was not observed for macrophages. In addition they speculated that the macrophages could be transfected by PCV present in phagocytosed lymphocytic apoptotic bodies.

This idea is very intriguing in conjunction with an interesting observation made by Tischer et al. (1995b): cells infected with PCV survived the mock infected cells for 16 passages and behaved as immortalized cells. They lost contact inhibition, formed colonies in agar, were anchorage independent, and had low serum requirement. The authors speculated that perhaps one of the viral proteins may have an affect on cells similar to large T antigen of SV40.

Porcine circoviruses are an unexplored but highly interesting group of viruses, which could attract more attention not only for their mode of pathogenesis in pigs, but also for implications in the area of human health, specifically the use of porcine heart xenotransplants. Their unique way of replication, the possible biological effects on the cells they infect, along with the capability to cross species barrier (Kiupel et al. 2001) makes them an important target for further research.

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