Circoviridea encapsidated DNA gives insights into viral-adopted eukaryotic lagging strand synthesis

Xaver Sidler[1], Titus Sydler[2] and Enrico Brugnera[1]
[1]Department of Farm Animals, Division of Swine Medicine, Vetsuisse Faculty, University of Zurich; [2]Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zurich.

Key words
PCV2 replication, PCV2 cell culture propagation, mapping of partial double stranded viral DNA

Aim of the study
The purpose of the financial support by FVO (Bern, Switzerland) was to provide start-up help for a Swiss National Science Foundation (SNSF) grant. This included finishing and submitting two manuscripts and further providing experimental groundwork for Aim1 of the SNSF grant. Prof. U. Hübscher (Vetsuisse Faculty, University of Zurich) noticed already in the 80s the insufficient replication of PCV2 in in vitro systems. Even after these many years it is still unclear how the single stranded (ss) DNA isoform of the encapsidated porcine circovirus type 2 (PCV2) will be converted in the host cell to the double stranded (ds) DNA isoform of the virus that is needed for viral propagation by the melting-pot rolling cycle replication. In fact, this step is so critical for viral propagation and yet it is enigmatic for the whole family of Circoviridae.

Material and methods
We used fixed and paraffin embedded pig tissue for oligonucleotide mediated in situ hybridization (ISH). The pig spleen tissue needed to be moderate to high infected with PCV2 by immunohistochemistry. To guaranty proper oligonucleotide priming we additionally designed a new PCR to determine preferential PCV2 ORF1 nucleotide sequence. Porcine circovirus type 1 free PK15 cells (a gift from Gordon Allan) were used to adjust propagation conditions for PCV2.

Results and significance
First, we confirmed the original observation that the putative primer begins around the translational start of ORF1 by oligonucleotide mediated pair wise viral genome walking. Second we mapped the approximately length of the putative primer to 35 to 50 deoxynucleotides. We analyzed these findings more in details in section 2.3.1.1b of the SNSF grant. Especially, as RNA signals are reduced in formalin fixed and paraffin embedded tissue sections described in Khaiseb et al. 2011.
Our other task was to explore cell culture conditions that would allow the in vitro production of PCV2 concentrations higher than 1010 virus particles per ml. The virus was needed for the 2. Aim of the SNSF grant. From regular cell culture up to 106 virus particles per ml could be produced. In the literature certain specific cell lines or chemicals had been used to get up to 109 virus particles per ml. We targeted with different chemicals host cell growth and also heat shock to provoke higher in vitro virus propagation. None of the combination was really effective although described as helpful for viral propagation. It turns out that really the input of recombinant PCV2 DNA and cell transfection confluence were the determining factors that gave up to 1011 viral particles/ml. We tested the viral capsid protection with DNase I digestion followed by a sybr-green based real-time PCR. Again we found that up to 1011 viral particles/ml might be harvested.
Having in mind our recent findings (manuscripts submitted) that Circovirdae is latent present in animals and humans and furtively reprograms T-cells it will be a priority to understand the viral replication in details. This will help to molecular design countermeasures specifically for the Circovirdae family members and generally for persistent viruses.

Publications, posters and presentations
Sydler, T.; Handke, M.; Weilenmann, R.; Lewis, F.I.; Sidler, X.; Brugnera, E. (…) Thymus reliably carries latent Circovirdae PCV2 infected cells during pig's ontogeny. (submitted).
Klausmann, S.; Sydler, T.; Summerfield, A.; Lewis, F.I.; Weilenmann, R.; Sidler, X.; Brugnera, E. (…) T-cell reprogramming through targeted CD4-coreceptor and T-cell receptor expression on maturing thymocytes by latent Circoviridae presence in thymus. (submitted).

Project *1.11.22*

Project duration *October 2012 – January 2014*