JOURNAL OF VIROLOGY, Mar. 1997, p. 2562–2566 Vol. 71, No. 3
0022-538X/97/$04.00+0

Mapping and Characterization of the Origin of DNA Replication of Porcine Circovirus

ANNETTE MANKERTZ,* FRAUKE PERSSON, JOACHIM MANKERTZ, GUNNAR BLAESS, AND HANS-JOERG BUHK

Fachbereich Gentechnik und Genetik, Robert Koch-Institut, 13353 Berlin, Germany

Received 2 April 1996/Accepted 26 November 1996

The origin of DNA replication of porcine circovirus (PCV) was mapped to a 111-bp fragment. On top of a hairpin, a nonanucleotide (TAGTATTAC) homologous to nonanucleotides of other viruses was identified. Mutation of this element abolishes replication. PCV may be related to a virus family characterized by single-stranded circular DNA genomes, rolling-circle replication, and homology of their *rep* proteins.

Porcine circovirus (PCV) was isolated in 1974 (29) from the porcine kidney cell line PK/15 (ATCC CCL 31). PCV is widespread in various pig populations: antibodies to PCV were found in about 85% of sera from pigs destined for slaughter (6, 14, 31, 34). No specific disease has been ascribed to it. PCV is a very small (17 nm in diameter), isometric, nonenveloped virus with a single-stranded circular DNA genome of 1,759 nucleotides (nt) (30). The sequence of PCV has been determined (3). Computer analysis has revealed six potential open reading frames (ORFs) (Fig. 1A). To our knowledge, PCV is the smallest virus that replicates autonomously in mammalian cells.

To date, little is known about the molecular basis of DNA replication of PCV in its host cells. No PCV-specific replication protein has been characterized yet. Previous studies have demonstrated the existence of subgenomic PCV particles (33). The so-called 5S form of the viral genome is a defective interfering particle. DNA from the defective interfering particle is single stranded, covalently closed, and of positive sense and represents a defined region of the viral genome. Our present investigations concerning the analysis of these subgenomic particles isolated from PCV-infected cell lines (1) revealed that all DNAs cloned from 5S particles, although of different sizes and with sequence deviations from the PCV genome, display similar structures (Fig. 1B): they contain a fragment corresponding to the PCV genome from approximately position 340 to position 990 with a central deletion of about 100 to 200 nt. Whether this structure reflects a functional requirement or is caused by the mode of formation of 5S DNA is not yet known.

To identify the origin of DNA replication, we constructed a set of clones in which the structure of the 5S DNA was mimicked and varied. PCV fragments were cloned into vector pUC19 (Table 1) and tested for replication activity. Ten micrograms of plasmid DNA was mixed with 1×10^6 to 5×10^6 PCV-infected porcine kidney cells (line PSM [32]) and pulsed with 250 μF and 230 V (Bio-Rad electroporation unit). Cells were cultured for 48 h with one change of medium. Viral DNA was reisolated subsequently by a standard lysis protocol (13) and analyzed for replication by a *Dpn*I assay (5). Ten micrograms of the extracted DNA was restricted with *Dpn*I and *Bam*HI. The restricted DNA was analyzed on a 1% agarose gel, vacuum blotted onto a positively charged nylon membrane (Boehringer Mannheim), UV cross-linked, and hybridized to digoxigenin (DIG)-labeled plasmid pUC19. Blots were visualized with CSPD [disodium 3-(4-methoxyspiro(1,2-dioxetane-3,2′-(5′-chloro)tricyclo(3.3.1.1^{3,7})decan)-4-yl)phenylphosphate] and NBT (4-nitro blue tetrazolium chloride)–X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) phosphate. Newly replicated plasmids are linearized by *Bam*HI but are resistant to *Dpn*I. The 773-bp insert of pOP1 (position 329 to 1101) showed replication activity detected by the *Dpn*I assay (Fig. 2) when the construct was transfected into PCV-infected cells. As expected, no *Dpn*I-resistant plasmid DNA was found when vector pUC19 was used as input DNA. When this experiment was repeated with PCV-free porcine kidney (PS) cells as a substrate for electroporation, no replication was observed for any tested plasmid (data not shown). This result demonstrates that the presence of PCV as a helper virus is necessary for replication of the investigated plasmids, presumably because it supplies one or more *trans*-acting replication factors. The finding further corroborates our previous observations that cloned 5S DNAs can replicate only in PCV-infected cell cultures (1).

In pOP2, the insert is shortened by a deletion of nt 528 to 616, corresponding to the central deletion found in the 5S DNAs. Plasmid pOP2 was also active in the transient-DNA-replication assay (Fig. 2). To investigate the role(s) of 5S DNA in replication, plasmids pOP3 and -4 were constructed (Fig. 1). The *Dpn*I assay revealed that plasmid pOP4, which carries the PCV fragment from position 617 to 1101 and matches the right part of 5S particles, had the capacity for replication, which was not observed with plasmid pOP3 (PCV position 329 to 526). When clones pOP5, -6, and -7, which combine the two fragments in different orders and orientations, were tested, no influence on the replication activity was found. Thus, nt 329 to 526, which correspond to the left fragment of 5S DNA, seem not to carry elements necessary for replication in the transient-replication assay. Since this fragment has been found in all 5S clones so far analyzed, it is reasonable to assume that it might serve a function other than in replication: perhaps it encodes a signal for packaging, the influence of which cannot be seen in the transient-replication assay, or its presence reflects the mechanism of formation of 5S molecules.

Plasmids pOP10 to pOP15 were constructed to further delimit the origin of DNA replication. The DNA replication assay revealed that the origin is located within the 111-bp *Bal*I-*Hae*III fragment (position 728 to 838 [Fig. 3]). This fragment is replicated in a transient-replication assay with PCV as a helper virus. Two smaller fragments (pOP13 and pOP15) did not show replication activity and may represent inactive origins

* Corresponding author. Mailing address: Robert Koch-Institut, Fachbereich 5.2, Nordufer 20, 13353 Berlin, Germany. Phone: (30)4547-2594. Fax: (30)4547-2598.

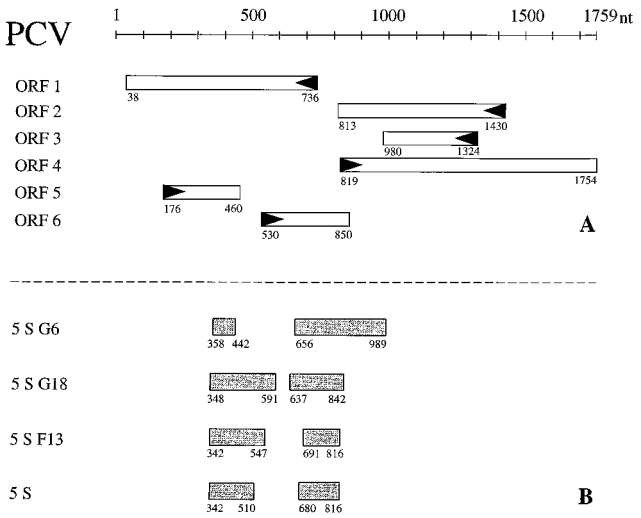

FIG. 1. Genome organization of PCV. The genome is represented in linear form. (A) Result of a computer-aided analysis for putative ORFs. ORFs are indicated by open boxes, and their orientations are indicated by the orientations of the triangles. The numbers below the boxes indicate the first and last nucleotides. (B) Locations of cloned subgenomic PCV DNA (5S DNA) with respect to the PCV genome.

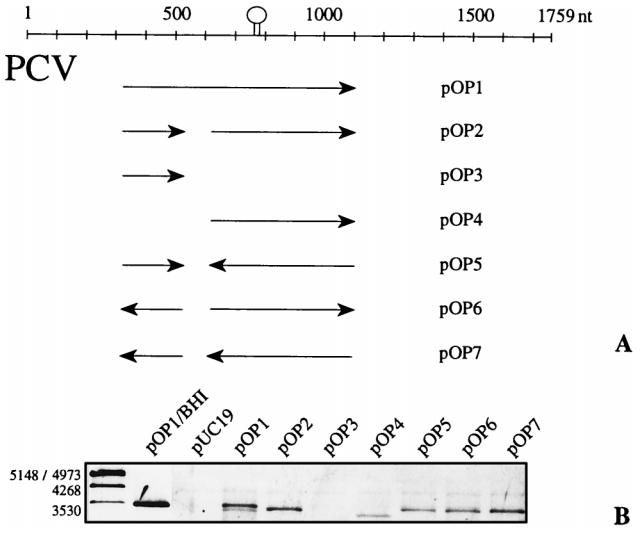

FIG. 2. Mapping of the replication origin. (A) Map of the genomic fragments of PCV tested in the transient-DNA-replication assay. The orientations of the inserted fragments relative to each other are indicated by the directions of the arrows. The location of the putative stem-loop structure is marked at the top. (B) Replication of plasmids pOP1 to pOP7 was tested after electroporation into PCV-infected PSM porcine kidney cells. Lane 1, DIG-labeled molecular size standard III (Boehringer Mannheim); lane 2, *Bam*HI-linearized input DNA plasmid pOP1 not subjected to *Dpn*I assay; lane 3, plasmid pUC19 electroporated into PSM cells; lanes 4 to 10, plasmids pOP1 to pOP7 electroporated into PSM cells. Fragment sizes are indicated on the left in base pairs.

in which control elements are deleted. This result is in agreement with sequence analysis of cloned 5S derivatives, which revealed that among the range of the right fragments of the 5S DNAs characterized, the shortest fragment (in clone 5S F13) spans from nt 691 to 816 (1).

The 111-bp fragment includes two conspicious sequence motifs which may serve as *cis*-acting elements in DNA replication (Fig. 4A). (i) A 6-bp motif (5′-CGGCAG-3′) is found three times adjacent to an inverted repeat. The role of this 6-bp motif is unclear; it may be used as a binding site by a putative PCV-encoded replication protein. This theory is supported by the finding that some 5S DNAs contain several short repeats which include the 6-bp motif (1). A related sequence (5′-GG TAGTAAGGTAG-3′) has been reported to be the binding site for the replication protein of tomato golden mosaic virus, (TGMV), a geminivirus (7). (ii) The sequence 5′-GAAGTGC GCTG-3′ is inversely repeated and may form a stable stem structure (ΔG° = 21.6 kcal/mol) (Fig. 4B). The sequence 5′-T AGTATTAC-3′ is found at the apex of the hairpin. Computer comparison revealed that this element connects PCV with two

TABLE 1. Plasmids derived from PCV

Plasmid	DNA fragment(s)[a]	Vector	Insertion site(s) of vector
pOP1	773-bp *Bst*XI-*Pst*I fragment, position 329–1101	pUC18	*Sma*I site
pOP2	485-bp *Hae*II-*Pst*I fragment, position 617–1101 (rev.); 198-bp *Bst*XI-*Hinc*II fragment, position 329–526 (rev.)	pUC18	*Hinc*II site (485-bp fragment) and *Sma*I site (198-bp fragment)
pOP3	198-bp *Bst*XI-*Hinc*II fragment, position 329–526	pUC18	*Sma*I site
pOP4	485-bp *Hae*II-*Pst*I fragment, position 617–1101 (rev.)	pUC18	*Hinc*II site
pOP5	485-bp *Hae*II-*Pst*I fragment, position 617–1101; 198-bp *Bst*XI-*Hinc*II fragment, position 329–526 (rev.)	pUC18	*Hinc*II site (485-bp fragment) and *Sma*I site (198-bp fragment)
pOP6	485-bp *Hae*II-*Pst*I fragment, position 617–1101 (rev.); 198-bp *Bst*XI-*Hinc*II fragment, position 329–526	pUC18	*Hinc*II site (485-bp fragment) and *Sma*I site (198-bp fragment)
pOP7	485-bp *Hae*II-*Pst*I fragment, position 617–1101; 198-bp *Bst*XI-*Hinc*II fragment, position 329–526	pUC18	*Hinc*II site (485-bp fragment) and *Sma*I site (198-bp fragment)
pOP9	Same as pOP4; *Pvu*II site introduced at position 765 of the PCV insert		
pOP10	345-bp *Nla*IV fragment, position 699–1043	pUC18	*Sma*I site
pOP11	263-bp *Sty*I-*Bst*NI fragment, position 722–984	pUC18	*Sma*I site
pOP12	111-bp *Bal*I-*Hae*III fragment, position 728–838	pUC18	*Sma*I site
pOP13	113-bp *Hph*I fragment, position 857–969	pGEM3Zf(+)	*Sma*I site
pOP14	190-bp *Sty*I-*Alu*I fragment, position 722–911	pUC19	*Sma*I site
pOP15	189-bp *Hga*I-*Bst*NI fragment, position 796–984	pUC19	*Sma*I site
pAN26	485-bp *Hae*II-*Pst*I fragment, position 617–1101	M13mp18	*Sma*I and *Pst*I sites
pAN26-mut5	Same as pAN26; *Pvu*II site introduced at position 765 of the PCV insert		

[a] All positions refer to the PCV genome. rev., reverse orientation.

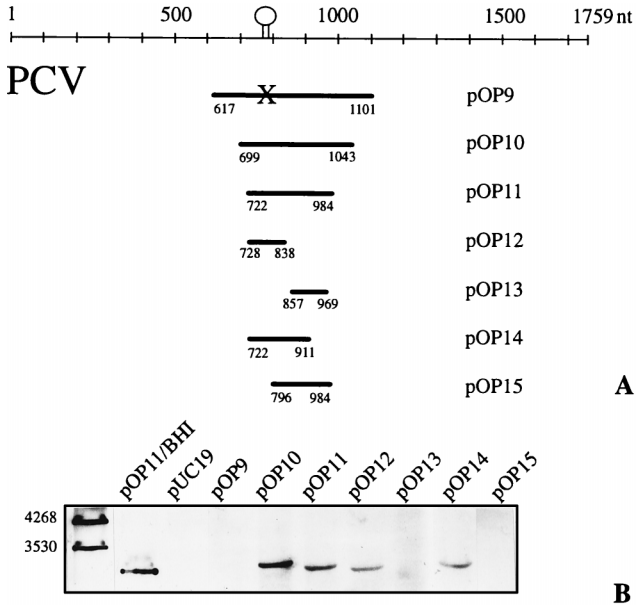

FIG. 3. Fine mapping of the replication origin. (A) Inserts of the tested plasmids. Plasmid pOP9 caries a mutation (X) that alters the 9-nt homology sequence found in PCV. The putative stem-loop is indicated on the map at the top. (B) Result of the transient-DNA-replication assay. Lane 1, DIG-labeled molecular size standard III; lane 2, *Bam*HI-linearized input DNA plasmid pOP11 not subjected to *Dpn*I assay; lane 3, plasmid pUC19 electroporated into PSM cells; lanes 4 to 10, plasmids pOP9 to pOP15 electroporated into PSM cells. Fragment sizes are indicated on the left in base pairs.

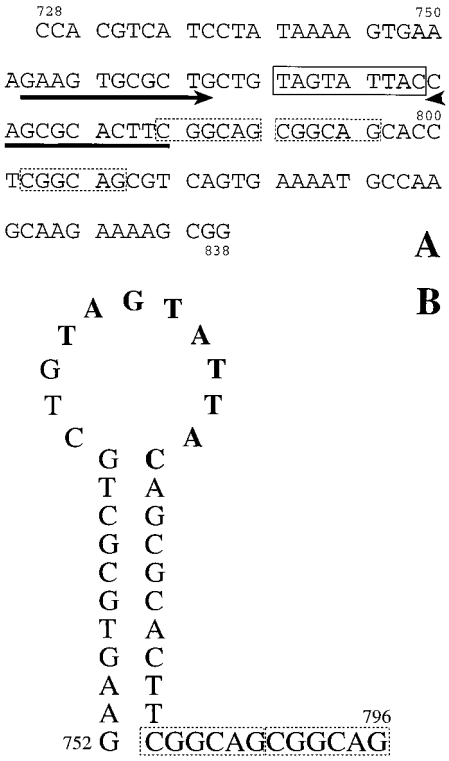

FIG. 4. Sequence of the replication origin of PCV. (A) Sequence of the 111-bp *Bal*I-*Hae*III fragment (position 728 to 838 of PCV), carrying the origin of DNA replication of PCV. The inverted repeat is underlined by arrows, the conserved nonanucleotide sequence is marked by a plain box, and the three 6-bp repeats are marked by dashed boxes. (B) Structural elements in the origin of PCV. The inverted repeat forming the potential hairpin structure is shown. The ΔG° for the stem-loop is −21.6 kcal/mol. The 9-nt consensus sequence is shown in boldface type, and two of the three 6-bp repeats found in the origin fragment are indicated by dashed boxes.

groups of plant viruses that are characterized as having single-stranded, circular DNA genomes. Their mode of replication has been described as rolling-circle-type replication (Table 2).

The family *Geminiviridae* comprises a unique group of DNA viruses. They consist of three subgroups that differ with respect to insect vector, host range, and genome organization (17). In the replication origins of all known geminiviruses, a sequence motif with the potential to form a hairpin structure and the recognition site of the essential viral replication protein is present. Within the loop of the hairpin an evolutionarily conserved sequence (5′-TAATATTAC-3′) is found. This sequence is very similar to the nonanucleotide found in PCV. Mutational analysis demonstrated that the 9-nt sequence in the geminivirus hairpin is an essential *cis*-acting element required for viral DNA replication (18, 23). Recent results also demonstrate that the hairpin structure is essential for geminiviral DNA replication (22). A rolling-circle mechanism with separate leading- and lagging-strand DNA synthesis steps is used for geminiviral replication. The initiation and termination sites for plus-strand replication have been mapped in vivo to the 9-nt motif conserved in all geminiviruses (11, 27). It has been shown that the virion sense DNA strand is nicked between nt 7 and 8 of this motif (TAATATT↓AC) during initiation of rolling-circle replication (12, 16, 26).

A similar motif is found in the genomes of some so-called subterranean clover stunt virus (SCSV)-like viruses: SCSV (2), coconut foliar decay virus (CFDV) (25), and banana bunchy

TABLE 2. Homology of the PCV nonanucleotide to nonanucleotides of other viral systems

Virus or replicon	Sequence	Genome structure	Mode of replication[a]	Reference
PCV	TAGTATTAC	Circular ssDNA[b]	?	This study
GV[c] subgroup I (e.g., MSV)	TAATATTAC	Circular ssDNA	RCR	17
GV subgroup III (e.g., TGMV)	TAATATTAC	Two components, circular ss DNA	RCR	17
CFDV	TAATACTAG	Circular ssDNA	RCR	25
CAV	TACTATTCC	Circular ssDNA	?	21
BBTV (component 1)	TATTATTAC	Six components, circular ssDNA	?	9
SCSV	(T/C)AGTATTAC	Seven components, circular ssDNA	?	2
ϕX174	TGATATTAT	Circular ssDNA	RCR	10
pC194	TGATAATAT	ssDNA plasmid	RCR	19

[a] ?, unknown; RCR, rolling-circle replication.
[b] ssDNA, single-stranded DNA.
[c] GV, geminivirus.

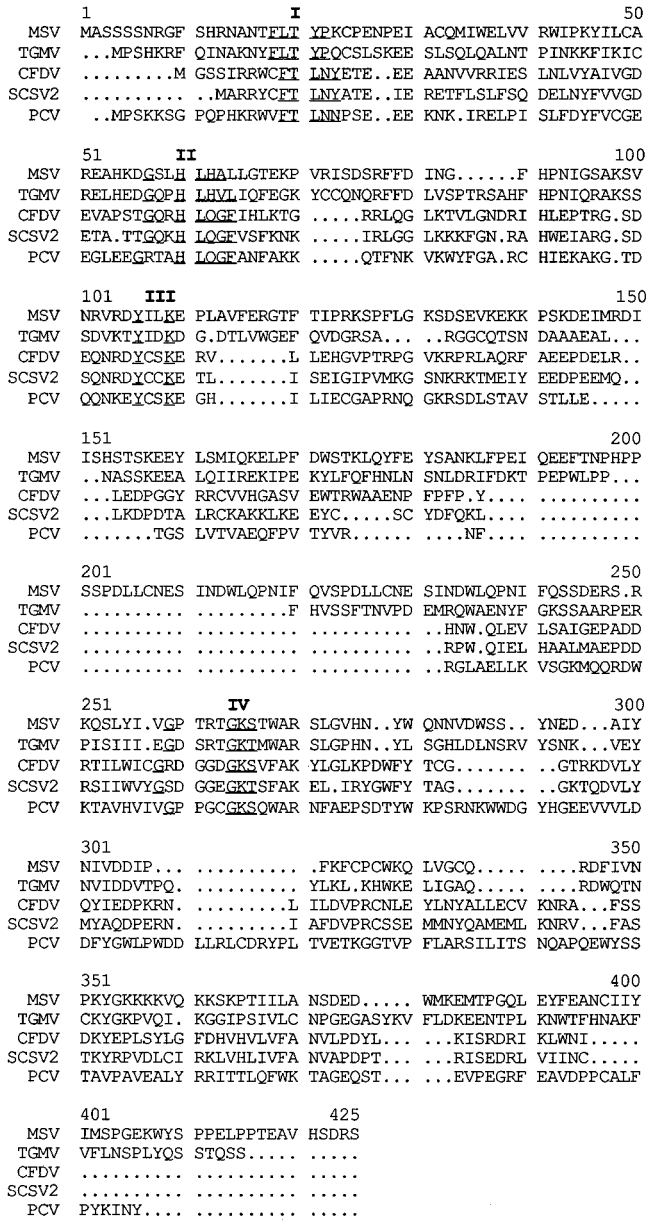

FIG. 5. Alignment of the replication proteins of single-stranded circular DNA viruses. Component 2 of the multipartite genome of SCSV (SCSV2) and the Nigerian isolate of MSV (2) were used. All four conserved motifs are indicated by roman numerals and underlining. The three domains typical for *rep* proteins involved in rolling-circle replication identified by Koonin and Ilyina (15) are indicated by I, II, and III, while IV marks the nucleotide binding site.

top virus (BBTV) (9). To date, only a single genome component of 1,291 nt has been identified for CFDV, while BBTV and SCSV are characterized by multipartite genomes consisting of at least six or seven distinct circular molecules, respectively, each of approximately 1 kb.

In chicken anemia virus (CAV) (4), a different 9-nt element is found. In contrast to the replicons mentioned above, the 9-nt element is not located at the top of a hairpin structure. The nonanucleotide sequence of PCV also resembles the gene A protein cleavage sites of *Escherichia coli* phage ϕX174 (5′-TG ATATTAT-3′) (10) and the gram-positive bacterial plasmid pC194 (5′-TGATAATAT-3′) (19). In ϕX174 and pC194, site-specific single-strand cleavage provides the 3′-OH terminus needed for the initiation of plus-strand synthesis, and the nick is therefore part of the plus-strand origin of replication.

TABLE 3. Evolutionary relationship of viral *rep* proteins

Virus	% Identity to[a]:				
	TGMV	MSV	CFDV	SCSV2	PCV
TGMV	100.00	32.35	14.71	15.36	14.58
MSV	32.35	100.00	14.44	15.64	10.86
CFDV	14.71	14.44	100.00	45.52	22.83
SCSV component 2	15.36	15.64	45.52	100.00	24.54
PCV	14.58	10.86	22.83	24.54	100.00

[a] Based on aligned regions of *rep* proteins of TGMV, MSV, CFDV, and SCSV and the ORF 4 product of PCV.

The homology to other replicons led us to the hypothesis that the hairpin and the conserved nonanucleotide element might play an essential role in PCV DNA replication. To test the significance of the nonanucleotide, a mutant of plasmid pOP4 in which the nonanucleotide was altered from **TA**GTA TTAC to **CT**GTATTAC was constructed by site-directed mutagenesis. A *Pvu*II site was introduced into plasmid pAN26 with an in vitro mutagenesis kit (U.S. Biochemical Corp.) and the oligonucleotide 5′-GCG CTG GTA ATA CAG CTG CAG CG-3′ (the newly generated *Pvu*II site is underlined, and mutations with respect to the PCV genome are in boldface type). After a screen for plasmids containing the newly generated *Pvu*II site, the mutation was verified by sequencing. The insert of the resultant plasmid, pAN26-mut5, was subcloned into vector pUC19 to construct plasmid pOP9. Transfected into PCV-infected cells, pOP9 showed a complete loss of replication activity as judged by the *Dpn*I assay (Fig. 3). Thus, the mutation of the nonanucleotide motif results in the inactivation of the origin of DNA replication of PCV. This observation demonstrates that the mutagenized nucleotides are essential for viral replication and underlines the importance of the 9-nt motif.

A second line of evidence for the association of PCV with geminiviruses and SCSV-like viruses is implied by the homology of their putative *rep* proteins. Although the *trans*-acting replication factor of PCV is not yet characterized, we propose that it is encoded by ORF 4. Comparison of the ORF 4 product's amino acid sequence to those of *rep* proteins of SCSV-like viruses (SCSV, BBTV, and CFDV) (2) and two representative geminiviruses (TGMV and maize streak virus [MSV] [20]) by the PILEUP program of the Genetics Computer Group sequence analysis package revealed homology around the nucleotide binding motif (Fig. 5). Homology of the putative nucleoside triphosphate binding domain **G**X_4**GKT**M**WAR**X_{28}**DD** (conserved residues in boldface type) within a group of 13 geminiviruses was shown previously (8). Mutational analysis demonstrated that this motif is an essential element for the activity of the *rep* proteins of the geminiviruses. A similar motif (**G**X_4**GKSQWAR**X_{29}**DD**) is present in the derived amino acid sequence of ORF 4 of PCV. Additionally, three other motifs which, according to Koonin and Ilyina (15), are typically associated with replication proteins involved in rolling-circle replication were found. Thus, the ORF 4 product was identified as the putative replication protein of PCV. When an evolutionary relationship between five *rep* proteins was calculated by the program TREE, we found that ORF 4 of PCV has more homology to the *rep* proteins of CFDV and SCSV (22 to 24%) than to the replication factors of MSV and TGMV (10 to 14%) (Table 3). The classification of PCV, psittacine beak and

feather disease virus (24), and CAV in the new family of circoviruses (28) based on structural and biochemical similarities must be considered carefully with respect to their homology: no replication protein in CAV has been characterized to date, and our analysis of the three proteins described earlier (21) did not reveal extensive homology to the putative *rep* protein of PCV. To our knowledge, the sequence of psittacine beak and feather disease virus is not yet available. Since the putative *rep* proteins of PCV, SCSV, and CFDV show striking sequence homology, it seems reasonable to discuss a relationship between these viruses. Based on the analysis of the origin of PCV and its putative *rep* protein, it is likely that PCV also replicates via a a rolling-circle-type mechanism. *cis*- and *trans*-acting factors essential for PCV as well as the replication mechanism are currently under investigation.

Nucleotide sequence accession number. The EMBL accession number of the PCV DNA sequence is Y09921.

The technical assistance of Annette Kluge and Roswitha Lorenz is gratefully acknowledged.

REFERENCES

1. **Blaess, G., R. Lorenz, J. Mankertz, I. Tischer, H.-J. Buhk, and A. Mankertz.** Cloning and sequencing of 5 S DNA, a subgenomic particle of porcine circovirus. Submitted for publication.
2. **Boevink, P., P. W. G. Chu, and P. Keese.** 1995. Sequence of subterranean clover stunt virus DNA: affinities with the geminiviruses. Virology **207:**354–361.
3. **Buhk, H.-J., I. Tischer, and M. A. Koch.** 1985. Cloning and sequencing of the porcine circovirus (PCV) genome. Zentralbl. Bakteriol. Mikrobiol. Hyg. Ser. A **260:**465.
4. **Claessens, J. A. J., C. C. Schrier, A. P. A. Mockett, E. H. J. M. Jagt, and P. J. A. Sondermeijer.** 1991. Molecular cloning and sequence analysis of the genome of chicken anaemia agent. J. Gen. Virol. **72:**2003–2006.
5. **DeLange, A. M., and G. McFadden.** 1986. Sequence-nonspecific replication of transfected plasmid DNA in poxvirus-infected cells. Proc. Natl. Acad. Sci. USA **83:**614–618.
6. **Dulac, G. C., and A. Afshar.** 1989. Porcine circovirus antigens in PK-15 cell line (ATCC CCL-33) and evidence of antibodies to circovirus in Canadian pigs. Can. J. Vet. Res. **53:**431–433.
7. **Fontes, E. P. B., P. A. Eagle, P. S. Sipe, V. A. Luckow, and L. Hanley-Bowdoin.** 1994. Interaction between a geminivirus replication protein and origin DNA is essential for viral replication. J. Biol. Chem. **269:**8459–8465.
8. **Hanson, S. F., R. A. Hoogstraten, P. Ahlquist, R. L. Gilbertson, D. R. Russell, and D. P. Maxwell.** 1995. Mutational analysis of a putative NTP-binding domain in the replication-associated protein (AC1) of bean golden mosaic virus. Virology **211:**1–9.
9. **Harding, R. M., T. M. Burns, G. Hafner, R. G. Dietzgen, and J. L. Dale.** 1993. Nucleotide sequence of one component of the banana bunchy top virus genome contains a putative replicase gene. J. Gen. Virol. **74:**323–328.
10. **Heidekamp, F., P. D. Baas, and H. S. Jansz.** 1982. Nucleotide sequences at the ϕ gene A protein cleavage site in replicative form I DNAs of bacteriophages U3, G14, and α3. J. Virol. **42:**91–99.
11. **Heyraud, F., V. Matzeit, M. Kammann, S. Schaefer, J. Schell, and B. Gronenborn.** 1993. Identification of the initiation sequence for viral-strand DNA synthesis of wheat dwarf virus. EMBO J. **12:**4445–4452.
12. **Heyraud-Nitschke, F., S. Schuhmacher, J. Laufs, S. Schaefer, J. Schell, and B. Gronenborn.** 1995. Determination of the origin cleavage and joining domain of geminivirus Rep proteins. Nucleic Acids Res. **23:**910–916.
13. **Hirt, B.** 1967. Selective extraction of polyomavirus DNA from infected mouse cell cultures. J. Mol. Biol. **26:**365–369.
14. **Horner, G. W.** 1991. Pig circovirus antibodies present in New Zealand. Surveillance (Wellington) **18:**23.
15. **Koonin, E. V., and T. V. Ilyina.** 1993. Computer assisted dissection of rolling circle DNA replication. BioSystems **30:**241–268.
16. **Laufs, J., W. Traut, F. Heyraud, V. Matzeit, S. G. Rogers, J. Schell, and B. Gronenborn.** 1995. In vitro cleavage and joining at the viral origin of replication by the replication initiator protein of tomato yellow leaf curl virus. Proc. Natl. Acad. Sci. USA **92:**3879–3883.
17. **Lazarowitz, S.** 1992. Geminiviruses: genome structure and gene function. Crit. Rev. Plant Sci. **11:**327–349.
18. **Lazarowitz, S. G., L. C. Wu, S. G. Rogers, and J. S. Elmer.** 1992. Sequence-specific interaction with the viral AL1 protein identifies a geminivirus DNA replication origin. Plant Cell **4:**799–809.
19. **Michel, B., and S. D. Ehrlich.** 1986. Illegitimate recombination occurs between the replication origin of the plasmid pC194 and a progressing replication fork. EMBO J. **5:**3691–3696.
20. **Mullineaux, P. M., J. Donson, B. A. M. Morris-Krsinich, M. I. Boulton, and J. W. Davies.** 1984. The nucleotide sequence of maize streak virus DNA. EMBO J. **3:**3063–3068.
21. **Noteborn, M. H. M., and G. Koch.** 1995. Chicken anaemia virus infection: molecular basis of pathogenicity. Avian Pathol. **24:**11–31.
22. **Orozco, B. M., and L. Hanley-Bowdoin.** 1996. A DNA structure is required for geminivirus replication origin function. J. Virol. **70:**148–158.
23. **Revington, G. N., G. Sunter, and D. M. Bisaro.** 1989. DNA sequences essential for replication of the B genome component of tomato golden mosaic virus. Plant Cell **1:**985–992.
24. **Ritchie, B. W., F. D. Niagro, P. D. Lukert, W. L. Steffens, and K. S. Latimer.** 1989. Characterization of a new virus from cockatoos with psittacine beak and feather disease. Virology **171:**83–88.
25. **Rohde, W., J. W. Randles, P. Langridge, and D. Hanold.** 1990. Nucleotide sequence of a circular single-stranded DNA associated with coconut foliar decay virus. Virology **176:**648–651.
26. **Stanley, J.** 1995. Analysis of African cassava mosaic virus recombinants suggests strand nicking occurs within the conserved nonanucleotide motif during the initiation of rolling circle DNA replication. Virology **206:**707–712.
27. **Stenger, D. C., G. N. Revington, M. C. Stevenson, and D. M. Bisaro.** 1991. Replicational release of geminivirus genomes from tandemly repeated copies: evidence for rolling circle replication of a plant viral DNA. Proc. Natl. Acad. Sci. USA **88:**8029–8033.
28. **Studdert, J. S.** 1993. Circoviridae: new viruses from pigs, parrots and chickens. Aust. Vet. J. **70:**121–122.
29. **Tischer, I., R. Rasch, and G. Tochtermann.** 1974. Characterization of papovavirus- and picornavirus-like particles in permanent pig kidney cell lines. Zentralbl. Bakteriol. Mikrobiol. Hyg. Ser. A **226:**153–167.
30. **Tischer, I., H. Gelderblom, W. Vettermann, and M. A. Koch.** 1982. A very small porcine virus with circular single-stranded DNA. Nature **295:**64–66.
31. **Tischer, I., W. Mields, D. Wolff, M. Vagt, and W. Griem.** 1986. Studies on epidemiology and pathogenicity of porcine circovirus. Arch. Virol. **91:**271–276.
32. **Tischer, I., D. Peters, R. Rasch, and S. Pociuli.** 1987. Replication of porcine circovirus: induction by glucosamine and cell cycle dependence. Arch. Virol. **96:**39–57.
33. **Tischer, I. and H.-J. Buhk.** 1988. Viral DNA from cells infected with porcine circovirus. Zentralbl. Bakteriol. Mikrobiol. Hyg. Ser. A **270:**280–287.
34. **Tischer, I., L. Bode, D. Peters, and B. Germann.** 1995. Distribution of antibodies to porcine circovirus in swine populations of different breeding farms. Arch. Virol. **140:**737–743.