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Nucleotide Sequence and Genome Organization of Canine Parvovirus

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The genome of a canine parvovirus isolate strain (CPV-N) was cloned, and the DNA sequence was determined. The entire genome, including ends, was 5,323 nucleotides in length. The terminal repeat at the 3′ end of the genome shared similar structural characteristics but limited homology with the rodent parvoviruses. The 5′ terminal repeat was not detected in any of the clones. Instead, a region of DNA starting near the capsid gene stop codon and extending 248 base pairs into the coding region had been duplicated and inserted 75 base pairs downstream from the poly(A) addition site. Consensus sequences for the 5′ donor and 3′ acceptor sites as well as promotors and poly(A) addition sites were identified and compared with the available information on related parvoviruses. The genomic organization of CPV-N is similar to that of feline parvovirus (FPV) in that there are two major open reading frames (668 and 722 amino acids) in the plus strand (mRNA polarity). Both coding domains are in the same frame, and no significant open reading frames were apparent in any of the other frames of both minus and plus DNA strands. The nucleotide and amino acid homologies of the capsid genes between CPV-N and FPV were 98 and 99%, respectively. In contrast, the nucleotide and amino acid homologies of the capsid genes for CPV-N and CPV-b (S. Rhode III, J. Virol. 54:630–633, 1985) were 95 and 98%, respectively. These results indicate that very few nucleotide or amino acid changes differentiate the antigenic and host range specificity of FPV and CPV.

Canine parvovirus (CPV) is a member of the autonomously replicating parvoviruses and is associated with enteritis and myocarditis in dogs. CPV-associated disease in dogs became predominant in 1978 (11) and is assumed to have arisen as a variant of feline parvovirus (FPV) (47). Information on the virus proteins (23) as well as on the DNA sequence (10, 41) verifies that there is a great deal of homology between FPV and CPV. Despite the close similarity, the two viruses can be distinguished on the basis of restriction enzyme mapping (28, 49), antigen cross-reactivity (35), and host range specificity (49). However, high passage of CPV in feline and canine cells can induce mutations which can be identified as variants from the parental strain (34). In addition, since 1978, new variants seem to have arisen in nature (35). Because these variants apparently arose so quickly, it is of growing concern to understand more about the mechanisms of virus replication and gene expression.

Autonomous parvoviruses have a single-stranded linear genome of approximately 5,000 nucleotides (nt). Nucleotide sequence (3, 10, 15, 41, 42, 46) and transcriptional mapping data (13, 21, 24, 25, 36, 48) have revealed several common features of parvoviruses. (i) There are two major open reading frames (ORFs). (ii) The ORF in the 3′ half of the genome encodes the nonstructural proteins, while the ORF in the 5′ half of the genome encodes the structural proteins. (iii) The nonstructural and structural genes are initiated from separate promotors. (vi) The mRNAs of the nonstructural and structural proteins have coterminal poly(A) addition sites (map position [mp] 94 to 96) and are spliced to allow alternate templates for protein synthesis.

The ability of parvoviruses to replicate in specific cells is dependent on both cellular and viral determinants. Host range- or strain-specific tropisms for both the fibrotrophic (p) and lymphotrophic (i) strains of the minute virus of mice (MVM) do not seem to occur at the penetration or uncoating stage but involve a mechanism occurring after synthesis of replicative form (RF) DNA (3, 43, 44). Thus, the strain-specific DNA determinant expressed by the variant virus dictates the host range specificity, since MVMi and MVMp are serologically identical and can bind to receptors on both restrictive and productive cell types (43). Strain-specific determinants can be observed in variant virus populations following high passage of the virus in transformed cell lines. For example, the 5′ untranslated region of H1 parvoviruses becomes reiterated when grown in NB cells (38, 39). A correlation between the DNA sequence variation observed in highly passaged parvovirus and changes in the virus DNA replication or gene expression patterns has not been demonstrated. Since high passage of parvoviruses induces changes in virus specificity and antigenic characteristics (34, 38, 39), it is of interest to compare the DNA sequence from a high-passage CPV isolate with sequences from related parvoviruses. We have cloned and sequenced a strain of CPV (isolated at Norden Laboratories, Lincoln, Nebr.) from a high-passage infection in dog kidney cells. This data and the available data for CPV (strain b) (41) and FPV (10) are compared. In addition, except for the terminal 5′ repeat, this is the first report for the entire CPV genome.

MATERIALS AND METHODS

Materials. All restriction enzymes and DNA modification enzymes were purchased from New England BioLabs, Inc., Beverly, Mass., or Bethesda Research Laboratories, Inc., Gaithersburg, Md. Isotopically labeled [α-^{32}P]dATP (>3,000 Ci/mM and [α-^{35}S]dATP (>1,000 Ci/mM) were purchased from Amersham Corp. Arlington Heights, Ill.

M13 vectors were obtained from Joachim Messing and were grown and prepared as described by Messing (30). Plasmid DNAs were purified by using standard cleared-lysis procedures and two bandings on CsCl (26). The plasmid DNAs used for cloning were pBR322, pHC624 (7; a gift from

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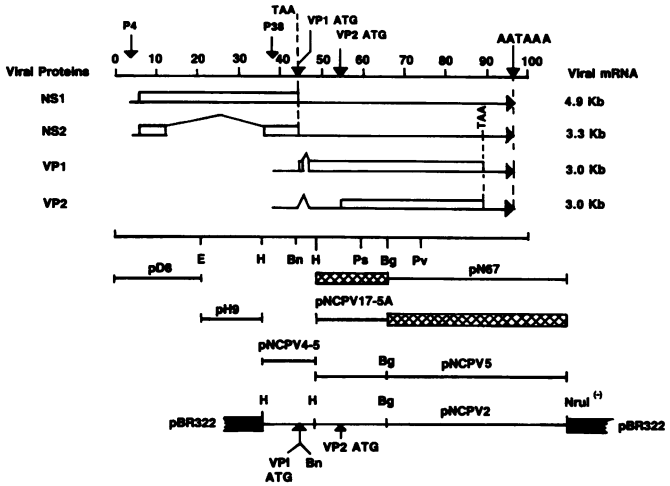

FIG. 1. Transcriptional and cloning map. The CPV genome is represented in the 3'→5' orientation and is divided by map coordinates. The major virion mRNAs and sizes are indicated on the right in kilobases, while the proposed protein each encodes are presented on the left. The important restriction enzymes in relation to map positions used in this study are indicated. E, *Eco*RI; H, *Hin*dIII; Bn, *Ban*I; Ps, *Pst*I; Bg, *Bgl*II; Pv, *Pvu*II. The bottom part of the figure depicts the regions cloned into various plasmid vectors. The cross-hatched area indicates the region of CPV-specific DNA in recombinant clones which displayed deletions or rearrangements. The *Nru*I$^{(-)}$ site is the former *Nru*I site used in ligation and is no longer a functional site. Cloned pD8 (blunt end to *Eco*RI of CPV RF DNA) was inserted between the *Eco*RI and *Pvu*II sites of pGEM 2 (29). Clone pH9 (*Eco*RI-*Hin*dIII of CPV RF DNA) was inserted between the *Eco*RI and *Hin*dIII sites of pHC624 (7).

Imre Boros, Szeged, Hungary) and pGEM-2 (29; Promega Biotec, Madison, Wis.).

Virus and cells. The Norden dog kidney cell line NL-DK1 (9) was grown in basal medium containing Earle salts and 5% fetal bovine serum. All virus passages were done on NL-DK1. The serum was previously irradiated with 2.5 Mrads of ionizing radiation to inactivate potential pathogens. CPV-N is a high passage of an isolate of CPV which displays a lowered virulence.

DNA isolation. RF DNA was extracted from infected cells by scraping the cells into medium and then centrifuging (SS34, 1,500 rpm, 1 min). The cell pellet was suspended in proteinase K (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) solution (50 mM Tris hydrochloride [pH 7.5], 1% sodium dodecyl sulfate, 10 mM EDTA, 750 μg of proteinase K per ml) and incubated for 4 to 5 h at 37°C. Sodium chloride was added to a final concentration of 1 M, and the mixture was incubated overnight at 4°C. The chromosomal DNA and particulate cellular debris were removed by centrifugation (SW41, 40,000 rpm, 30 min, 4°C). Low-molecular-weight DNA and RNA were ethanol precipitated from the supernatant and then extracted with phenol-chloroform-isoamylalcohol (25:25:1). The aqueous phase was layered onto a 5 to 20% linear sucrose gradient and centrifuged (SW28, 24,000 rpm, 16 h, 4°C). Fractions containing the RF DNA (monitored on a 1% agarose gel by electrophoresis in Tris borate buffer) were ethanol precipitated and suspended in deionized H_2O.

Recombinant DNA and transformation. Purified RF DNA was digested with appropriate restriction enzymes, and the individual bands were separated on either 1% agarose gels (HMT SeaKem; FMC Corp., Marine Colloids Div., Rockland, Maine) in TBE buffer or 0.6% agarose gels (LMT SeaPlaque; FMC Corp.) in TAE buffer (26). Bands were electroeluted from the high-melting-point agarose and purified by extracting twice with butanol (saturated with 0.15 M NaCl and 10 mM Tris, pH 7.6) and twice with phenol-chloroform-isoamyl alcohol (25:25:1). The DNA was ethanol precipitated and added to standard ligation reactions (26). The ligated DNA was transformed into DH5 *Escherichia coli* cultures as described by Hanahan (22). Low-molecular-weight DNA or DNA in low concentrations was ligated directly from the low-melting-temperature agarose (17). DNA bands were excised from the gel and melted at 65°C for 10 min. The volume was measured, and water was added to give a 0.1% solution of melted agarose. Better results were obtained when the agarose did not gel during the ligation or transformation procedures. Ligation reactions were done in 200-μl aliquots by adding 178 μl of the melted agarose solution, 20 μl of 10× ligation buffer (22), and 2 μl of DNA ligase and incubating the reaction mixtures at 14°C for 16 h. Transformations were performed by adding 100 μl of the ligation mixture to 200 μl of C600 competent cells prepared as described by Crouse et al. (17). All transformation mixtures were plated onto L plates containing 100 μg of ampicillin per ml. Colonies were picked and screened for CPV-specific inserts by dot blot hybridization by using ^{32}P-labeled purified viral DNA (26).

Cloning strategy for the CPV genome. All designations for the virus genome orientation are drawn with the 3' end of the minus-strand DNA to the left (2).

The strategy for cloning the entire genome required digesting purified CPV RF DNA with *Hin*dIII and *Eco*RI (mp 35 and 47.8 for *Hin*dIII and mp 21 for *Eco*RI; Fig. 1). *Hin*dIII cleaves the RF DNA into three fragments (28). One fragment contains the major portion of the nonstructural-protein-coding region (NS1 and NS2) (mp 0 to 35). The central fragment (mp 35 to 47.8) contains the proposed ATG (mp 44) for the VP1 mRNA and the proposed major splice junctions for VP1 and VP2 mRNAs. The third fragment contains the major coding region of the VP1 and VP2 proteins (mp 47.8 to 100).

The 3' end (mp 0 to 35) fragment was cleaved with *Eco*RI (mp 21) to generate an additional fragment. The 3'-most terminal fragment (mp 0 to 21) was cloned into the *Eco*RI-to-*Pvu* II site of pGEM 2 (Fig. 1, pD8). The *Eco*RI-*Hin*dIII fragment (mp 21 to 35) was cloned in pHC624 (6) (Fig. 1, pH9). The central *Hin*dIII fragment (mp 35 to 47.8) was easily cloned into the *Hin*dIII site of pBR322 (Fig. 1, pNCPV4-5). The 5'-end fragment was cloned into the *Hin*dIII-to-*Nru*I (blunt-end site at mp 972) site of pBR322 (Fig. 1, pN67 and pNCPV17-5A).

Sequencing CPV DNA mp 21 to 100. CPV-specific DNAs isolated from pH9, pNCPV4-5, and pNCPV5 (combination of noncrosshatched regions of pNCPV17-5A and pN67 (Fig. 1) were digested with restriction enzymes (*Hin*dIII, *Hin*cII, *Nco*I, *Aha*I, *Hpa*II, *Rsa*I, and *Alu*I) to create random sets of cloned fragments representing the entire region. The restriction fragments were then cloned into M13mp18 or M13mp19 double-stranded RF DNA by using combinations of appropriate restriction enzymes. Ligated DNA was transformed into JM101 cells, and white plaques were picked for single-stranded template preparation. The template DNAs were then sequenced by using the dideoxy chain termination method (45) and a universal forward primer. Template DNAs were isolated, and the sequence was determined on a minimum of two separate gels. Regions of overlap were determined by computer and direct analysis. Restriction

enzyme sites at all junctions were identified from the sequencing gels.

Sequencing of the CPV DNA mp 0 to 21. Plasmid pD8 contains mp 0 to 21 of CPV in a pGEM 2 plasmid. The CPV insert plus flanking pGEM 2 DNA was isolated on a *Taq*I fragment and cloned in both orientations in M13mp19. The 3′ exonuclease activity of T4 DNA polymerase was then used to produce a series of overlapping clones from single-stranded DNA templates by using complementary DNA oligomers to form specific restriction enzyme sites (18). The DNA was then transformed into JM101, and template DNA was prepared for sequencing.

Ambiguities arising in the sequence by using the dideoxy chain termination method on the 3′-end palindromic sequence were resolved by using Maxam and Gilbert sequencing (27). Complete resolution of the sequence required the incorporation of up to 40% (final) formamide in the sequencing-gel mix.

BAL 31 digestion. Plasmid DNA was digested with single-site restriction enzymes and then purified by phenol-chloroform extraction and ethanol precipitation. The DNA was suspended in water, and 3 to 6 U of BAL 31 enzyme was added in the appropriate buffer (26). The reactions were terminated in 1% sodium dodecyl sulfate–10 mM EGTA [ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetra-acetic acid] at 67°C. The DNA was phenol-chloroform extracted, and *Xba*I linkers were added in the presence of 8 U of T4 DNA ligase. After ligation, *Xba*I was added to digest the linker DNA, and the ends were ligated into covalently closed circles before transformation into *E. coli*.

Computer program and analysis. The DNA sequence analysis was performed by using the Beckman Microgenie on an IBM PC AT and the University of Wisconsin programs with a DEC VAX computer. A sonic digitizer (Science Accessories Associates) was used with the Microgenie software to transfer sequences to a computer file.

RESULTS

DNA sequence of the 5′ untranslated region. By digesting CPV RF DNA with *Eco*RI and *Hin*dIII, fragments were generated which contained the blunt end of RF DNA and an internal cohesive restriction enzyme site. The strategy was to directionally clone these fragments into pBR322. However, very few of these clones were stable in various strains of *E. coli* by using a single vector. Three separate cloning vectors (pBR322, pGEM 2, and pHC624) were necessary to generate stable clones in transformation-competent *E. coli*. Two plasmids, pN67 and pNCPV17-5A (cloned in pBR322), were originally thought to contain an intact insert (mp 45.8 to 100) but in fact contained deletions or rearrangements when analyzed by restriction enzymes (Fig. 1). The nonrearranged or nondeleted regions of pN67 and pNCPV17-5A were combined to form pNCPV5. Plasmid pNCPV5 was then combined with pNCPV4-5 to reconstruct the entire VP1- and VP2-coding region in pBR322 (Fig. 1, pNCPV-2).

The region of the genome from mp 47.8 (*Hin*dIII) to the end of the genome was sequenced from clone pNCPV5. This clone contained a region larger than anticipated in the 5′ untranslated region by restriction enzyme analysis. Partial sequence data and restriction mapping confirmed that about 400 to 500 nt had been duplicated in the 5′ untranslated region starting 51 base pairs (bp) from the *Hae*III site (mp 94). By using the clone pNCPV2 which contains the entire VP1- and VP2-coding sequences, BAL 31 enzyme treatment was used to generate a nested set of clones which contained progressively shorter regions of the repeated 5′ untranslated region. An *Ava*I site at nt 1425 of pBR322 was used to initiate the BAL 31 digestion. The *Ava*I site lies 453 bp downstream from the *Nru*I site used as the blunt-end cloning site. *Xba*I linkers were added to generate a unique restriction enzyme site to aid in the manipulation of the pNCPV-2-coding region. Two clones (p7-1 and p14-14) were isolated from the transformation of BAL 31-treated DNA, which by size were estimated to have the potential full-length, unreiterated 5′ untranslated genomic DNA (Fig. 2). DNA sequence data of p7-1 and p14-14 revealed that they differed in size by 255 bp (Fig. 2). Clone p7-1 contains 58 nt immediately after the *Hae*III site which was not vector DNA. Clone p14-14 contains an additional 255 bp, which begins 51 bp after the *Hae*III site. This 255-bp region does not display any characteristics of the 5′ palindromic sequences contained within any of the other parvoviruses. Instead, this 255-bp region is a repeat of part of the capsid-coding sequence upstream of the stop codon (nt 4289 to 4543) (Fig. 2; see Fig. 4). The 255-bp region is a perfect repeat, except at nt position 4295, where a C is inserted to give the repeat at nt 5067 one extra base.

The untranslated region also displayed DNA sequence reiteration observed in other parvoviruses (12). One 62-nt repeat begins at nt 4514 and ends at nt 4574 and flanks the stop codon at nt 4538. The second 62-nt repeat begins at nt 4575, ends at nt 4636, and contains a 1-base insertion at nt 4593 and a transition (C to T) at nt 4603 (see Fig. 4). Another direct 62-nt repeat begins at nt 4701 and is repeated three times, ending at nt 4886.

Sequence of the 3′ end. The 3′ end of the genome was sequenced from clone pD8 which contains a 1.1-kilobase insert (mp 0 to 21) between the *Eco*RI and *Pvu*II site of pGEM 2 (Fig. 1). Since no convenient restriction sites were available to allow easy subcloning into M13 vectors, the whole 1.1-kilobase insert plus some pGem 2-flanking regions were cloned into M13mp19. Nested sets of the single-stranded M13-cloned templates were made, starting from the *Eco*RI site by using the T4 DNA polymerase 3′ exonuclease technique (18). Two regions of the DNA in the T structure of the palindrome could not be resolved by the normal dideoxy chain termination method (Fig. 3). These two areas were resolved by using a combination of Maxam and Gilbert sequencing and increased denaturing conditions of the gel. The 3′ palindromic sequence resembles that of the rodent parvoviruses (12). The total length was 118 nt, with 92 nucleotides making up the stem structure and 26 nt making up the T structure (Fig. 3). Two regions of the stem structure were conserved between rodent parvoviruses and CPV-N: nt 1 to 12 of the rodent and nt 1 to 12 of CPV-N (11 of 12 match) and nt 38 to 48 of CPV match nt 34 to 44 of rodent parvoviruses (10 of 10 match) (12). The T structure of CPV-N displayed some divergence, with 18 of 26 nt matching sequence and location with 18 of 27 nt in rodent parvoviruses (nt 51 to 62, 65 to 67, and 69 to 71 of CPV-N match nt 49 to 60, 62 to 64, and 66 to 68 of rodent parvoviruses, respectively) (12; Fig. 3).

Sequencing mp 21 to 94. Recombinant DNA clones pH9, pNCPV4-5, and pNCPV2 were used to determine the sequence of the major body of the genome (Fig. 1). The CPV-specific inserts were isolated from these recombinants and digested with multiple enzymes (see Materials and Methods). The digested fragments were cloned into M13mp18 and M13mp19 to generate random sets of clones. Computer analysis was used to identify regions of overlap, and all restriction enzyme junctions were positively identified on

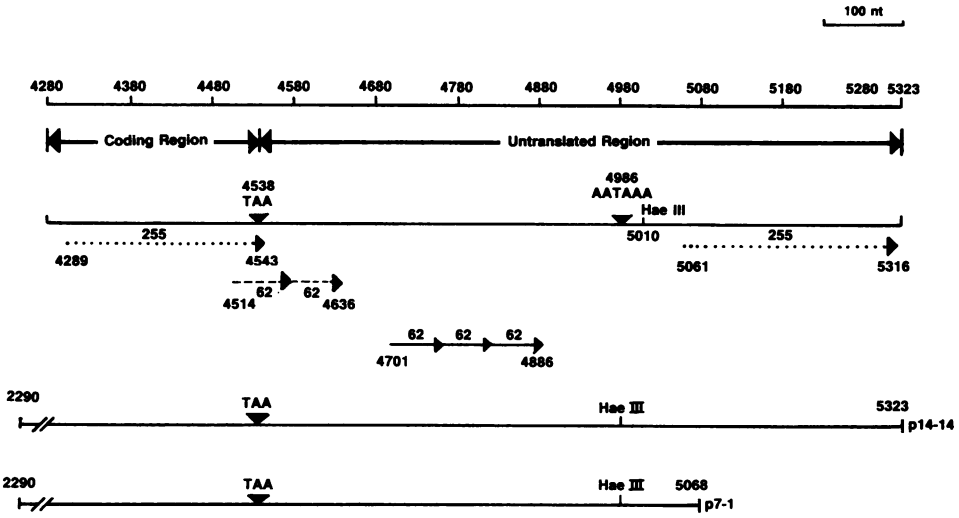

FIG. 2. Detailed map of the 5′ untranslated region of genomic DNA. Clones p14-14 and p7-1 end in the 3′-terminal region. p14-14 and p7-1 were constructed by using BAL 31 as described in the text.

sequencing gel autoradiograms. Unlike the terminal regions, the internal sequence contained a high percentage of A and T residues, which reduced the ambiguities and problems associated with compressions on denaturing gels. The sequence and single-letter amino acid translation of CPV-N (mp 0 to 94) is shown in Fig. 4.

Genome organization and assignment of CPV genes. The potential coding domains for both the C strand (plus polarity) and the V strand (minus polarity) are shown in Fig. 5. There are two predominant ORFs, both occurring in frame 2 of the C strand (Fig. 5). The first ORF, A (668 amino acids) is probably the coding domain for the nonstructural proteins (NS1 and NS2; 16, 24, 46). ORF B (722 amino acids) is most likely the coding domain for the capsid proteins (VP1 and VP2; 16, 24, 41, 46). There are several smaller ORFs in both the C and V strands. Whether these small ORFs are of any significance is not known. However, one small ORF (ORF S) in frame 1 of the C strand is the end of the 255-nt duplication from the coding domain ORF B (described above). This 255-nt region is no longer open in frame 2 of the C strand, but instead, 201 nt are now open in frame 1 (Fig. 4 and 5). An ATG at nt 5110 and a TAA at nt 5311 would allow for a small peptide 34 amino acids long. In addition, a TATA box occurs at nt 5026, placing a potential cap site at nucleotide position 5056, 4 nt from the start of the 255-nt duplication. The TATA at nt 5026 also has a potential E and A region (see below; Table 1), making it a candidate for a eucaryotic promoter sequence. By using comparative analysis of previously published data (10, 24, 25, 40, 41) with the data described here, a more precise assignment of the CPV-coding region was made.

A computer search for possible promoter regions was done by using the information on eucaryotic promoters described by Bensimhon et al. (5). These features include a stable enabler region (E) approximately 100 bp upstream from the cap site, a G+C-rich activator region (A) approximately 50 to 75 bp upstream of the cap site, and the TATA box which usually lies about 30 bp upstream from the cap site (5). Eight possible promoter sites were characterized by using a consensus search for the sequence TATAA (Table 1). Two promoters, one at mp 3.5 (P3.5) (Fig. 4, TATAAAA, nt 188) and a second at mp 38 (P38) (Fig. 4, TATAAAT, nt 1990) contained all the appropriate promoter components. The P3.5 promoter of CPV-N is analogous in map position and sequence to other parvovirus promoters and probably initiates transcription of the large ORF A for the CPV nonstructural genes (15, 16, 42). The P38 promoter most likely initiates transcription of ORF B for the structural genes (VP1 and VP2) and is homologous to P38 promoters described for other parvoviruses (3, 25, 36, 40, 42). The remaining six potential promoters were also analyzed for the presence of E and A regions. Three of these promoters (P28, P45, and P94) contained all of the components, while three lacked the E or A region (Table 1).

Two possible poly(A) addition sites were found by using AATAAA as the consensus sequence. One is in the major coding body of ORF A (nt 1580), and the other is located 448 bp downstream from the ORF B stop codon at nt 4538.

The potential 5′ donor sites (consensus sequence, $^{A}_{C}AGGT^{A}_{G}AGT$) and the 3′ acceptor sites [consensus sequence, $(Py)_6XCAGG^{C}_{T}$] used as mRNA splice junctions (14) are listed in Table 2 and mapped in Fig. 5. Six possible 5′ donor sites were identified. The sequence of these donor sites represents regions with greater than 65% homology (six of nine base pairs match) with the consensus sequence, allowing only one mismatch. Three of the donor sites are located in ORF A (nt 294, 306, and 508). One donor lies immediately upstream of the proposed ATG start codon for VP1 (nt 2277), while the remaining two (nt 2314 and 4375) are in ORF B (Fig. 4 and Table 2).

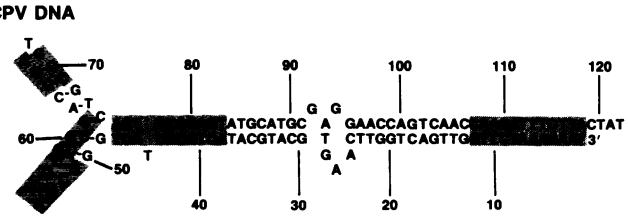

FIG. 3. Structure of the 3′ palindromic DNA. Clone pD8 in plasmid pGem 2 was used to determine the sequence of the 3′ end of the genome. Shaded areas are regions which are homologous to rodent parvovirus sequences.

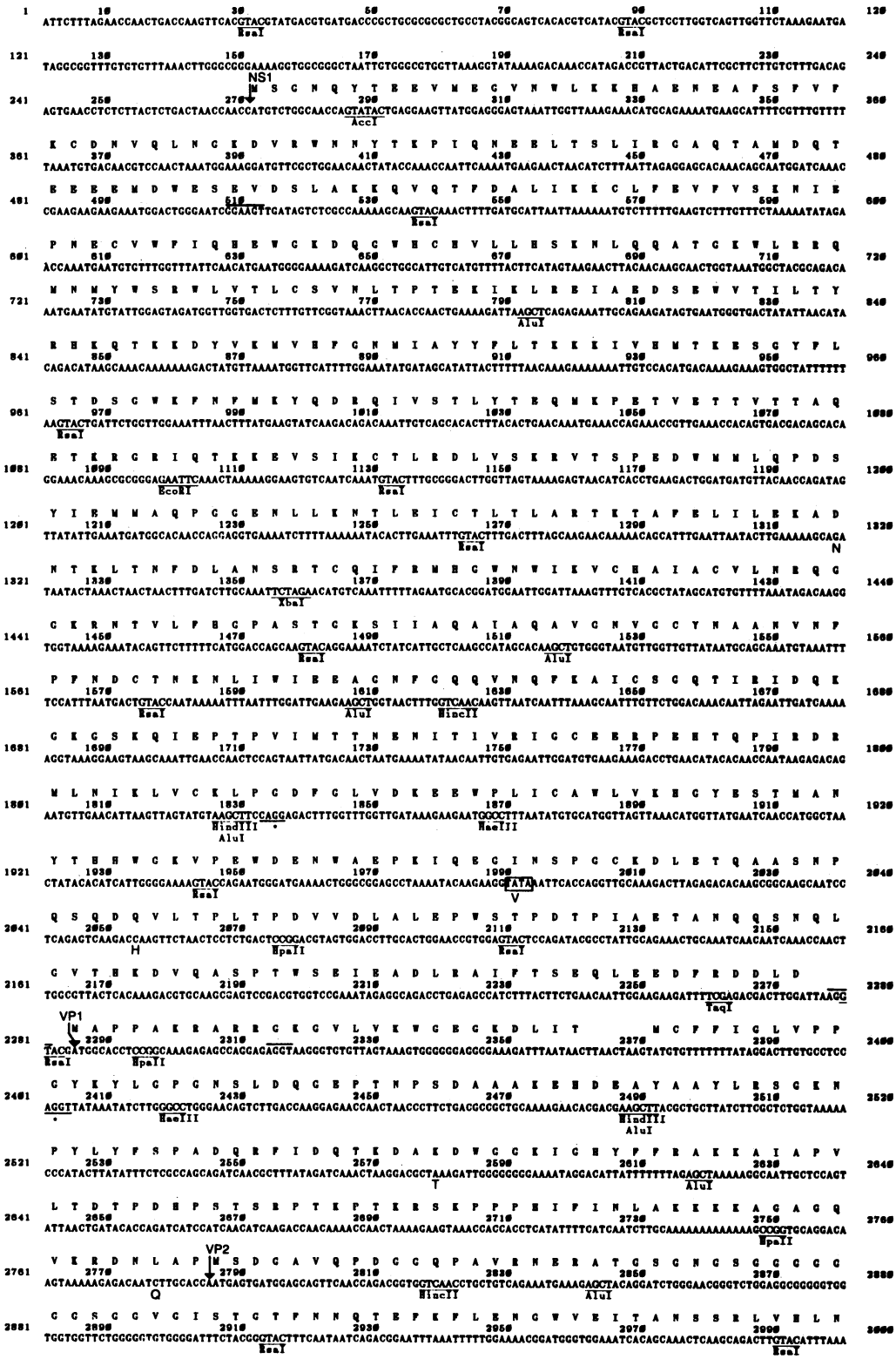

FIG. 4.—*Continued.*

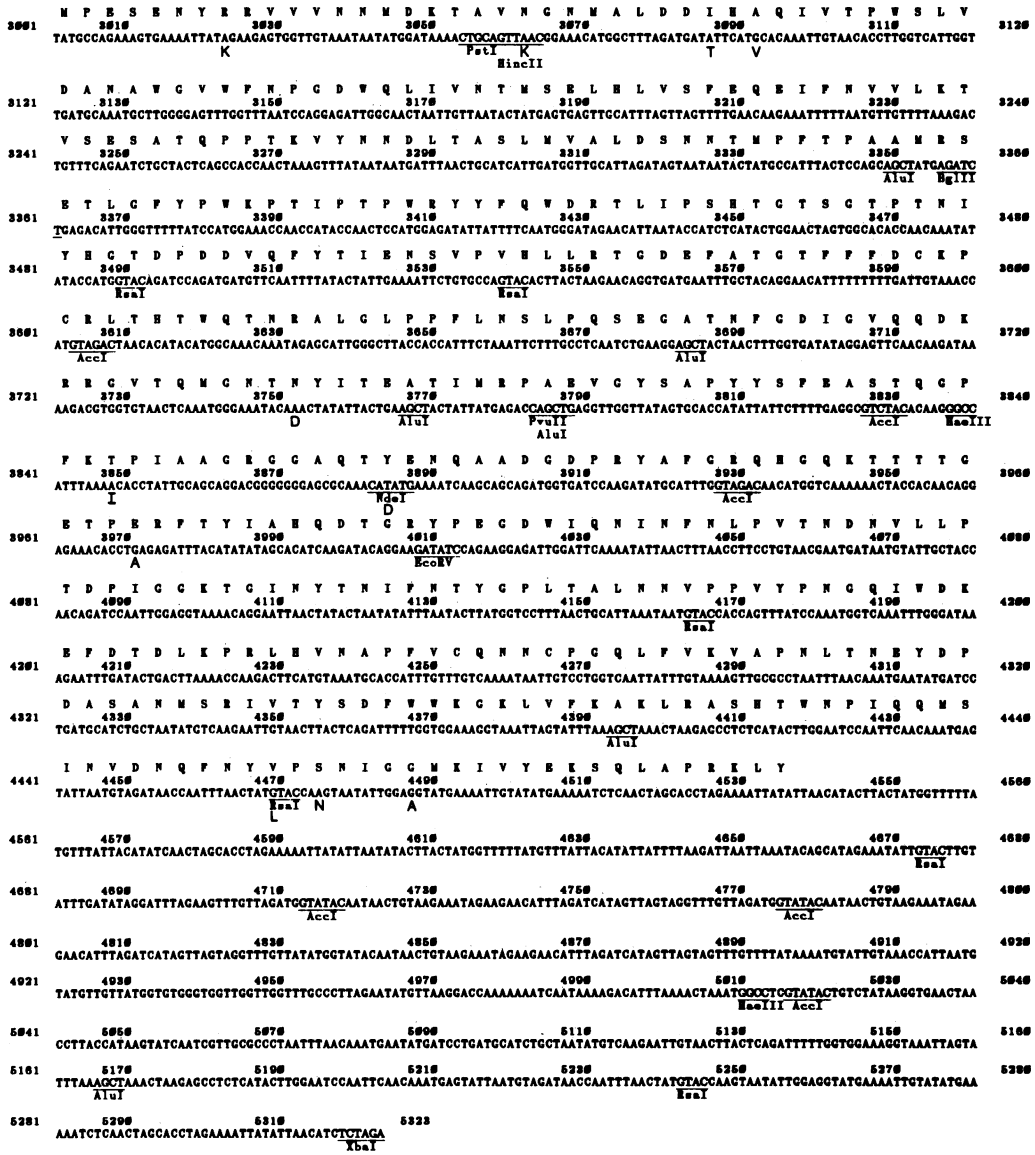

FIG. 4. DNA sequence of the CPV complementary strand. The single-stranded nucleotide sequence of the C strand is shown with the one-letter amino acid translation. Below the CPV nucleotide sequence is the one-letter amino acid letter indicating the changes that occur between FPV (10) and CPV. Overlined regions indicate the proposed mRNA 5′ donor splice junction, and the 3′ acceptor splice junctions are underlined.

A total of 16 possible 3′ acceptor regions were identified (Table 2); however, only 5 of these sites demonstrated better than 65% homology (8 of 12 match) with the consensus sequence (14) and retained the core CAGG region (Table 2). Two of the five high-homology 3′ acceptor regions are in ORF A, and three are in ORF B (Fig. 5).

Sequence homology of CPV and other parvoviruses. The homology between CPV-N, CPV-b, FPV, and other parvoviruses is shown in Table 3. CPV-N by both amino acid and nucleotide homology is more analogous to FPV than to CPV-b. Homology with MVM and H1 is lower, and very little homology exists between CPV-N and the human parvovirus B19 (46). Certain stretches of DNA sequence within the nonstructural (NS1) coding region are highly conserved between all parvoviruses except B19 human parvovirus (amino acids 352 to 516 of CPV have 90% or greater homology with amino acids 350 to 514 of H1 and amino acids 399 to 564 of MVM parvovirus). Incomplete sequence data of the FPV genome did not allow us to compare amino acid homologies of the nonstructural genes of CPV-N and FPV. However, the sequence for the structural genes was available and is compared in Table 4 and Fig. 6. By hydropathy analysis (University of Wisconsin computer programs), CPV-N and FPV show very little difference between their respective structural protein-coding regions.

The amino acid differences among FPV, CPV-N, and CPV-b are shown in Table 4. Only by close analysis can any of these changes be readily apparent in the hydropathy chart (Fig. 6). There are only 13 amino acid changes between the CPV-N and FPV capsid genes (Table 4 and Fig. 4), while

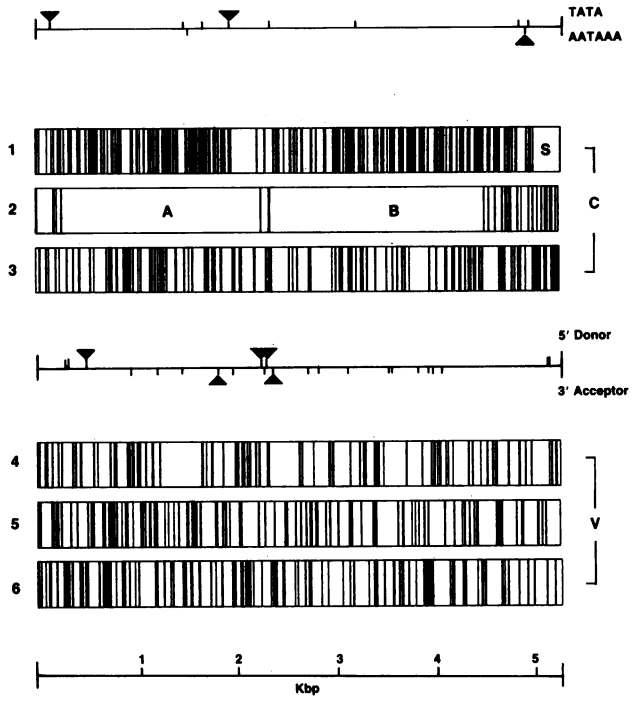

FIG. 5. Genomic organization of the viral (V) (minus-polarity) and complementary (C) (plus-polarity) strand. Each line designates the stop codon position of each frame in the viral and complementary strands. At the top of the figure is the map of the consensus sequence search for promoter-type elements (TATA) (upper) and poly(A) addition signal (AATAAA) (lower). Arrowheads indicate the major TATA and AATAAA elements proposed for CPV mRNA synthesis. The center part of the figure shows the map for the consensus sequence search for the 5′ donor ($^{A}_{C}$AGGT$^{A}_{G}$AGT) (upper) and the 3′ acceptor [(Py)$_6$XCAGG$^{C}_{T}$) (lower) splice junctions. Arrowheads indicate the proposed major mRNA splice sites.

there are 22 changes between FPV and CPV-b. A total of 10 of the amino acid changes are identical for both CPV-N and CPV-b (Table 4, footnote *c*).

The nucleotide differences among these viruses are also very limited; only 47 nucleotides differ between FPV and CPV-N (mp 20 to 96 were compared). These results indicate that even high-passage virus is homologous in sequence to FPV or CPV-b. The most prominent differences are located in the 5′ untranslated region and are changes in reiteration of base sequence rather than changes in the sequence itself (Fig. 2 and 4).

DISCUSSION

The entire sequence of the CPV-N genome was determined from clones made from RF DNA. The ends of the RF DNA were cloned by using an internal restriction enzyme site and the blunt end of the RF DNA in a directional cloning protocol. The 5′ half of the genome was cloned into the *Hin*dIII and *Nru*I sites of pBR322, and the 3′ half was cloned in the *Eco*RI and *Pvu*II sites of pGem 2. Restriction map and DNA sequence analysis of the 3′ end sequence indicated that the 3′-end palindromic sequence was cloned intact and resembles the T-stem structure of other rodent parvoviruses (12). The 5′-end palindromic sequence was not found intact in any of the clones sequenced. Instead, a 255-nt sequence representing the carboxy terminus of the VP1-VP2-coding region had been duplicated 51 nt downstream from the *Hae*III site. The duplication may have arisen as an anomaly of the cloning in *E. coli*, which was needed to stabilize the clone. However, this sequence reiteration may be caused by high passage of CPV-N in canine cells. At least two forms of RF DNA (differing by approximately 500 bp) have been observed in high-passage CPV-infected cells (data not shown). The 255-nt sequence contains 201 nt open in frame 1 (Fig. 5), and an upstream TATA sequence at 5026 (40 nt downstream of the poly(A) addition sequence (Table 1) could serve as a promoter for this small ORF S. The significance of the 255-bp duplication and its potential coding capacity were not examined for this report, but it is interesting that FPV also contains a similar TATA-type sequence 40 nt downstream from the poly(A) addition signal (10). By comparison, H1 parvovirus contains a TATT sequence 34 nt downstream of the poly(A) addition sequence, and MVM contains a TATT sequence 37 nt downstream of the poly(A) addition sequence (4, 42). A more striking homology is a G-rich region which lies upstream of the poly(A) sequence. In CPV-N the sequence GGGTGGTTGG occurs starting 49 nt upstream of the poly(A) addition sequence. The same sequence occurs upstream of the poly(A) addition sequence for MVM, H1, FPV, and CPV-b at a distance of 50, 48, 49, and 49 nt, respectively (4, 10, 41, 42). This sequence is located approximately 80 to 100 nt upstream from the potential TATA sequence described above and is similar to the E region for eucaryotic promotors (5). Recombinant clone pNCPV-2 originally contained 400 to 500 additional nt which were removed by BAL 31 treatment. Clones p7-1 and p14-14, generated in this manner, were chosen for examination because they approximated the expected length of the full genome of CPV. It is now of interest to examine the sequence of the 400- to 500-bp duplicated region to determine if any additional ORFs exist and specifically to determine if the poly(A) addition sequence is duplicated to provide ORF S with a potential polyadenylation signal.

Although the complete genomic sequence has not been determined for CPV-b and FPV, the available data suggest that the coding domains for the nonstructural and structural genes are in the same reading frame (46). Based on computer

TABLE 1. Analysis of potential CPV-N promoter sequences

Promoter[a]	Enabling sequence[b]	Activator[b]	TATA[c]
P3.5	GGGCGGGA (145; −73)	GGCGGGCA (159; −59)	TATAAAA (188)
P28	GGACCAGC (1469; −99)	GCTGTGGG (1517; −51)	TATAATG (1538)
P32			TATAACA (1738)
P38	GGGGAAAA (1935; −85)	GGGCGGAG (1965; −55)	TATAAAT (1990)
P45	GGGGGGAG (2337; −97)	GTGCCTCC (2393; −41)	TATAAAT (2404)
P63		GCCACCAA (3262; −44)	TATAATA (3278)
P91			TATAAAA (4894)
P94	GGTGTGGG (4932; −124)	GGCCTCGT (5010; −46)	TATAAGG (5026)

[a] The map position of the promoter is indicated.
[b] The first number in parentheses is the position of the sequence. The second number is the position from the cap site.
[c] The TATA is assumed to be 30 bp upstream from the mRNA cap site. The number in parentheses is the position of the sequence.

TABLE 2. Location of consensus 3′ acceptor and 5′ donor sites[a]

5′ Donors		3′ Acceptors	
Sequence	Location (nt)	Sequence	Location (nt)
AGG-AAGT	294	GACAGCACAGGA	1079
AGG-GAGT	306	GCACAACCAGGA	1224
GG-AAGT	508	GCAAGTACAGGA	1482
AAGGTACG	2277	AAGCTTCCAGGA	1833
AGGTAAG	2314	AATTCACCAGGT	2001
AAGGTAAA	4375	AGAGAGCCAGGA	2308
		GTGCCTCCAGGT	2400
		GCCGGTGCAGGA	2754
		AGAGCTACAGGA	2847
		TTTAATCCAGGA	3153
		CTAAGAACAGGT	3555
		TTTGCTACAGGA	3573
		ATTGCAGCAGGA	3861
		ACCACAACAGGA	3957
		CAAGATACAGGA	4002
		GGTAAAACAGGA	4104

[a] The consensus sequence for the 5′ donor was $\frac{A}{C}AGGT\frac{A}{G}AGT$; the consensus sequence for the 3′ acceptor was $(Py)_6XCAGG\frac{C}{T}$ (14). Of the nine possible nucleotides in the 5′ donor the computer searched for a minimum of one mismatch in at least six of nine nucleotides. No loop-outs were allowed. The computer searched for a minimum of three mismatches with no loop-outs in at least 7 of the 12 possible nucleotides in the 3′ acceptor. The regions underlined represent nucleotide-matching consensus sequences.

analysis of the potential reading frames in both the C and V strands of the virus, the coding domains do in fact lie within the same frame of CPV-N (Fig. 5). ORF A contains a promoter element as expected at mp 3.5 (nt 188) and a poly(A) addition site at nt 1580 (Fig. 5). The purpose of this poly(A) site is not known; however, there were no new mRNA transcripts detected on Northern gels (RNA blots) by using CPV-specific probes (data not shown). It is predicted that all the CPV mRNAs have a coterminus at the poly(A) site at nt 4985. ORF B (Fig. 5) contains a promoter element at nt 1990 (mp 38). These two promoters (mp 3.5 and 38) are analogous to those observed in other parvoviruses (3, 15, 16, 25, 36, 40, 42). The abundance of other promoter sites within the CPV sequence was not expected, although multiple promoter sites do exist in bovine parvovirus (15).

The consensus sequence computer search for potential 5′ donor and 3′ acceptor sites for CPV mRNA revealed sites which are analogous to the locations mapped for MVM mRNA splice junctions. Recent reports suggest that MVM utilizes three separate splice junctions, using all three ORFs of the C strand to regulate synthesis of the four main mRNAs (24, 33). By analogy to MVM, the predicted mRNA species for CPV-N may include an mRNA which uses a 5′ donor at nt 2314 and a 3′ receptor at nt 2400 (Table 2 and Fig. 5). This RNA would be analogous to MVM mRNA R1, which is translated to make the NS1 protein. The 5′ donor at nt 508 (nt 508 showed greater homology to the 5′ consensus se-

TABLE 3. Percent nucleotide and amino acid homology between CPV-N and other parvoviruses

Virus strain (relevant mp and reference)	% Homology with CPV-N		
	Nucleotide	Amino acid	
		NS1 and 2	VP1 and 2
CPV 780929 (mp 33–95; 41)	95	ND[a]	98
FPV (mp 20–96; 10)	98	99	99
B19 human	22	23	20
H1	62	73[b]	53
MVMi	47	73[b]	54
MVMp	47	73[b]	53

[a] ND, Not determined in publication.

[b] Specific regions between mp 20 and 30 of the NS1 protein of H1, MVMi, MVMp, and CPV-d are highly homologous (90%). This homology is not observed with the human strain.

TABLE 4. Comparison of amino acid charges between proposed capsid protein sequence of FPV, CPV-N, and CPV-b

Amino acid no.[a]	FPV	CPV-N	CPV-b 780929[b]
690	Asn	Asn	Lys
741	Thr	Lys[c]	Lys
807	Gln	Leu[c]	Leu
887	Asn	Asn	Ser
888	Tyr	Tyr	Glu
889	Lys	Arg	Lys
890	Deleted	Deleted	Asp
891	Deleted	Deleted	Arg
902	Lys	Asn[c]	Asn
910	Thr	Ile[c]	Ile
912	Val	Ala[c]	Ala
927	Gly	Gly	Asp
1118	Gln	Gln	Pro
1122	Arg	Arg	Lys
1132	Asp	Asn[c]	Asn
1164	Ile	Thr	Ile
1165	Pro	Pro	Deleted
1166	Ile	Ile	Deleted
1176	Asp	Tyr	Asp
1195	Gln	Gln	Glu
1220	Ala	Glu[c]	Glu
1242	Thr	Thr	Ile
1311	Val	Val	Leu
1371	Leu	Val[c]	Val
1373	Asn	Ser[c]	Ser
1377	Val	Gly[c]	Gly
1389	Pro	Pro	Gly

[a] Amino acid number is indicated in Fig. 6. The initiation codon for VP1 is amino acid 671, and for VP2 it is amino acid 810. The nucleotide numbers for VP1 and VP2 start codons are 2285 and 2786, respectively (Fig. 4).

[b] The sequence of the CPV-b capsid genes published previously was that of strain 780929 (Cornell University) (41).

[c] Amino acid changes common to both CPV strains.

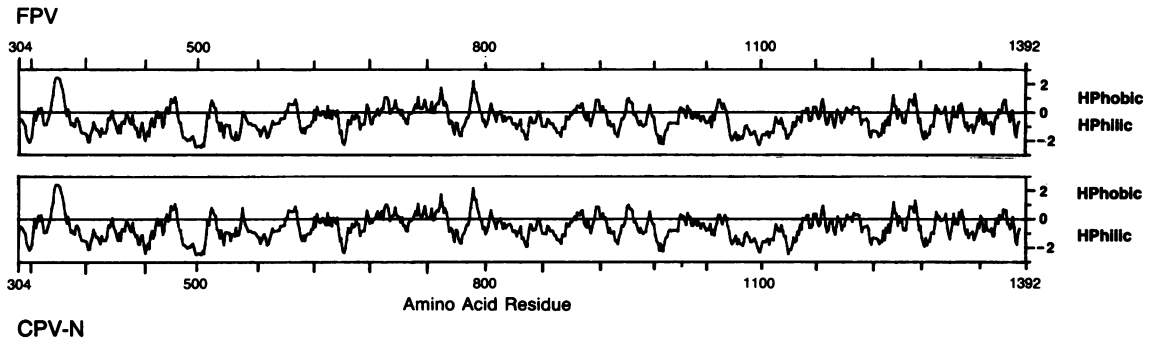

FIG. 6. Hydropathy analysis of CPV and FPV. The amino acid sequence for CPV and the available sequence for FPV were compared for hydrophobicity (HPhobic) and hyodrophilicity (HPhilic) by using University of Wisconsin computer programs. The available sequence for FPV is equivalent to number 304 of the CPV NS1 gene. There is a 6-nt untranslated region between the stop codon for NS1 (amino acid 670) and the start of VP1 (amino acid 672) (Fig. 4).

quence than did nt 532 of MVM [23]) and the 3′ acceptor at nt 1833 for CPV-N (Table 2) are likely candidates for NS2 mRNA coordinates. Unlike MVM, these messages must splice within the same frame since no other ORF exists on the C strand. The splice used to generate the mRNA expressing VP2 in CPV-N most likely utilizes the donor at nt 2277 and the acceptor at nt 2400 (41). This would be analogous to mRNA R3 of MVM (24) and is designed to remove the ATG of VP1 at nt 2285 (Fig. 4). VP1 mRNA for CPV probably uses the same 3′ acceptor at nt 2400 but uses an alternate 5′ donor at nt 2314 to allow the VP1 ATG to remain intact (Fig. 4 and 5). The MVM VP1 mRNA (R3′) uses splice junctions at similar map positions but also splices across two reading frames (24). These predicted splice junctions can be verified by S1 mapping and cDNA sequencing. The significance of additional donor and acceptor sites (Table 2) is not known. However, since no new RNA species have been detected by using Northern blot analysis (data not shown), these additional donor and acceptor sites are probably not major splice sites.

The total DNA sequence data for CPV-b, CPV-N, and FPV are translated into percent homologies in Table 3. CPV-N displays greater than 98% homology with FPV in both nucleotide and amino acid sequence (Table 3 and Fig. 4). The rodent parvoviruses display various degrees of homology at the nucleotide level; however, the amino acids match more closely (Table 1). Certain regions within the NS1 protein are highly conserved between FPV, CPV, and the rodent parvoviruses. Amino acids 352 to 516 of CPV are 90% homologous with amino acids 350 to 514 of H1 parvovirus and amino acids 399 to 564 of the MVM strains. Human B19 parvovirus displayed very little homology at the nucleotide level or in genomic organization. However, a small consensus region in the NS1 gene of B19, MVM, and adeno-associated virus type 2 is also partially conserved in CPV (amino acids 346 to 373) and is postulated to be homologous to a domain in ATPase-like proteins of other viruses (1). Homology comparisons for bovine parvovirus were not done, but probably bovine parvovirus is also distantly related to CPV and FPV (15).

By using the available sequence data for FPV (11) and CPV-N, a hydropathy chart was generated. The N-terminal region of the FPV nonstructural gene region is not available, but the C-terminal portion is represented by amino acids 304 to 670 (Fig. 6). Amino acid 304 is the position assigned to the proposed NS1 protein in ORF A of CPV-N (Fig. 5) and represents the first available amino acid position from the available FPV sequence (10). The entire structural gene region is represented by amino acids 672 to 1392 (Fig. 6). The hydrophilic and hydrophobic character of the FPV and CPV are almost identical. Within the capsid gene region of FPV and CPV-N (Fig. 6, amino acids 672 to 1392) there are only 13 amino acid differences (Table 4). Some of these amino acid differences could cause a charge difference, but only close analysis will reveal any change in hydropathy (Table 4 and Fig. 6). There are 22 amino acid changes between FPV and CPV-b in the capsid gene region (Table 4), and 10 of these amino acids are shared with CPV-N. Four amino acid changes occur between nt 3054 and 3783 (*Pst*I-*Pvu*II) of CPV-N, which has been indicated as an important region for determining antigen and host range specificity of the virus (34). CPV-b has seven amino acid changes in this region, four of which are the same as those in CPV-N. Since the capsid genes are important for antigenic and infectivity characteristics (31, 32), these amino acid changes may be of great significance for determining a possible mechanism for capsid formation and species specificity (8, 19, 20).

The homology comparison and genomic organization of CPV-N and FPV demonstrate their close genetic identity. However, the two viruses can be distinguished by antigenic and host range specificity. Therefore, it is evident that only a few amino acid differences are critical for altering the specificity of these viruses. The only other apparent difference between CPV-N and FPV is in the reiteration of DNA sequence within the 5′ untranslated region of the genome. There are two separate and unrelated 62-nt repeats in the 5′ untranslated region of CPV-N. One flanks the stop codon at nt 4538 and has been reported in other parvovirus sequences (3, 10, 41). The other 62-nt repeat (repeated three times) is located 65 nt downstream from the end of the first repeat (Fig. 2) and ends 100 bp upstream from the poly(A) addition site (nt 4986). Beginning 66 nt downstream from the end of the poly(A) addition site (nt 5061), a 255-bp duplication (nt 4289 to 4543) occurs. The purpose of this duplication is not known, but details of the region are described above. Reiteration of DNA sequence in the 5′ untranslated region has been reported for variants of H1 parvovirus. These H1 variants were isolated by high passage of the virus in a transformed cell line (simian virus 40-transformed human newborn kidney cells). A reiteration of a 55-nt region upstream of the poly(A) addition site (12, 38, 39) is comparable in map positions to the reiterations observed in the 5′ untranslated region of CPV-N. DNA sequence from CPV-b (41) does not have the same reiteration as CPV-N does. This

may be the result of repeated passage, since CPV-N was sequenced from a high passage of a CPV isolate. As stated above, these reiterations and duplications may have arisen as a result of the cloning protocol. The significance of these reiterations may be important in determining the events that occur in the formation of altered or variant viruses. The reiteration of the 5′ untranslated region may be utilized differently during the packaging event. By using specific probes from cloned DNA, it will be possible to determine if high-passage RF DNA contains the same reiterations.

The strong homology of CPV-N with FPV supports the hypothesis that CPV arose as a variant of FPV. If it is considered that CPV is an FPV variant replicating in dog cells (a semipermissive host), then the reiteration of the 5′ untranslated DNA of high-passage CPV is not unlike that observed for variant H1 parvovirus. CPV, as a variant of FPV, may undergo similar genomic changes after multiple exposures to dogs in the natural population. Reports have already been published indicating that variant forms of CPV can be isolated from the natural dog population. Some variants can be distinguished from previous isolates made in 1978 (35). We have evidence (unpublished) which suggests that high-passage CPV has a unique distribution of capsid proteins in purified virions. Our laboratory is interested in the dependence of virus capsid processing on DNA template determinants as a possible mechanism for inducing altered antigenic or host range characteristics.

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LITERATURE CITED

1. **Anton, I. A., and D. P. Lane.** 1986. Non-structural protein 1 of parvoviruses: homology to purine nucleotide using proteins and early proteins of papovaviruses. Nucleic Acids Res. **14**:7813.
2. **Armentrout, R., R. Bates, K. Berns, B. Carter, M. Chow, D. Dressler, K. Fife, W. Hauswirth, G. Hayward, G. Lavelle, S. Rhode, S. Strauss, P. Tattersall, and D. Ward.** 1978. A standard nomenclature for restriction endonuclease fragments, p. 523–526. *In* D. Ward and P. Tattersall (ed.), Replication of parvoviruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
3. **Astell, C. R., E. M. Gardiner, and P. Tattersall.** 1986. DNA sequence of the lymphotropic variant of minute virus of mice, MVM(i), and comparison with the DNA sequence of the fibrotropic prototype strain. J. Virol. **57**:656–669.
4. **Astell, C. R., M. Thomson, M. Merchlinsky, and D. C. Ward.** 1983. The complete DNA sequence of minute virus of mice, an autonomous parvovirus. Nucleic Acids Res. **11**:999–1018.
5. **Bensimhon, M., J. Gabarro-Arpa, and L. Reiss.** 1983. Physical characteristics in eucaryotic promoters. Nucleic Acids Res. **11**:4521–4540.
6. **Biggin, M. D., T. J. Gibson, and G. F. Hong.** 1983. Buffer gradient gels and ^{35}S label as an aid to rapid DNA sequence determination. Proc. Natl. Acad. Sci. USA **80**:3963–3965.
7. **Boros, I., G. Posfai, and P. Venetianer.** 1984. High-copy-number derivatives of the plasmid cloning vector pBR322. Gene **30**:257–260.
8. **Both, G. W., C. H. Shi, and E. D. Kilbourne.** 1983. Hemagglutinin of swine influenza virus: a single amino acid change pleiotropically affects viral antigenicity and replication. Proc. Natl. Acad. Sci. USA **80**:6996–7000.
9. **Brown, A. L., E. V. Davis, and W. H. Beckenhauer.** 1968. Studies of an established canine kidney cell line. Cornell Vet. **58**:593–613.
10. **Carlson, J., K. Rushlow, I. Maxwell, F. Maxwell, S. Winston, and W. Hahn.** 1985. Cloning and sequence of DNA encoding structural proteins of the autonomous parvovirus feline panleukopenia virus. J. Virol. **55**:574–582.
11. **Carmichael, I. F., and L. N. Binn.** 1981. New canine infections. Adv. Vet. Sci. **1981**:1–37.
12. **Carter, B. J.** 1984. Variant on defective interfering parvovirus, p. 209–258. *In* K. I. Berns (ed.), The parvoviruses. Plenum Publishing Corp., New York.
13. **Carter, B. J., C. A. Laughlin, and C. J. Marcuss-Sekura.** 1984. Parvovirus transcription, p. 209–258. *In* K. I. Berns (ed.), The parvoviruses. Plenum Publishing Corp., New York.
14. **Cech, T. R.** 1983. RNA splicing: three themes with variations. Cell **34**:713–716.
15. **Chen, K. C., B. C. Shull, E. A. Moses, M. Lederman, E. R. Stout, and R. C. Bates.** 1986. Complete nucleotide sequence and genome organization of bovine parvovirus. J. Virol. **60**:1085–1097.
16. **Cotmore, S. F., and P. Tattersall.** 1986. Organization of nonstructural genes of the autonomous parvovirus minute virus of mice. J. Virol. **58**:724–732.
17. **Crouse, G. F., A. Frischant, and H. Lehrach.** 1983. An integrated and simplified approach to cloning into plasmid and single stranded phages. Methods Enzymol. **101**:78–89.
18. **Dale, R. M. K., B. A. McClure, and J. P. Houchins.** 1985. A rapid single-stranded cloning strategy for producing a sequential series of overlapping clones for use in DNA sequencing: application to sequencing the corn mitochondrial 18S RDNA. Plasmid **13**:31–40.
19. **Dietzschold, B., W. H. Wunner, T. J. Wiktor, A. D. Lopes, M. Lafon, C. L. Smith, and H. Koprowski.** 1983. Characterization of an antigenic determinant of the glycoprotein that correlates with pathogenicity of rabies virus. Proc. Natl. Acad. Sci. USA **80**:70–74.
20. **Dorner, A. J., J. P. Stoye, and J. M. Coffin.** 1985. Molecular basis of host range variation in avian retroviruses. J. Virol. **53**:32–39.
21. **Green, M. R., and R. G. Roeder.** 1980. Transcripts of the adeno-associated virus genome: mapping of the major RNAs. J. Virol. **36**:79–92.
22. **Hanahan, D.** 1983. Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol. **166**:557–580.
23. **Johnson, B.** 1984. Parvovirus proteins, p. 259–295. *In* K. I. Berns (ed.), The parvoviruses. Plenum Publishing Corp., New York.
24. **Jongeneel, C. V., R. Sahli, G. K. McMaster, and B. Hirt.** 1986. A precise map of splice junctions in the mRNAs of minute virus of mice, an autonomous parvovirus. J. Virol. **59**:564–573.
25. **Labieniec-Pintel, L., and D. Pintel.** 1986. The minute virus of mice P_{39} transcription unit can encode both capsid proteins. J. Virol. **57**:1163–1167.
26. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol. **65**:499–560.
28. **McMaster, G. K., J.-D. Tratschin, and G. Siegl.** 1981. Comparison of canine parvovirus with mink enteritis virus by restriction site mapping. J. Virol. **38**:368–371.
29. **Melton, D. A., P. A. Krieg, M. R. Rebagliati, T. Maniatis, K. Zinn, and M. R. Green.** 1984. Efficient *in vitro* synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. Nucleic Acids Res. **12**:7035–7056.
30. **Messing, J.** 1983. New M13 vectors for cloning. Methods Enzymol. **101**:20–78.
31. **Mitra, S., C. E. Snyder, R. C. Bates, and P. T. Banerjee.** 1982. Comparative physiochemical and biological properties of two strains of Kilham rat virus, a non-defective parvovirus. J. Gen. Virol. **1**:43–54.
32. **Molitor, T. W., H. S. Joo, and M. S. Collett.** 1985. KBSH parvovirus: comparison with porcine parvovirus. J. Virol. **5**: 257–263.

33. **Morgan, W. R., and D. C. Ward.** 1986. Three splicing patterns are used to excise the small intron common to all minute virus of mice RNAs. J. Virol. **60**:1170–1174.
34. **Parrish, C. R., and L. E. Carmichael.** 1986. Characterization and recombination mapping of an antigenic and host range mutation of canine parvovirus. Virology **148**:121–132.
35. **Parrish, C. R., P. H. O'Connell, J. F. Evermann, and L. E. Carmichael.** 1985. Natural variation of canine parvovirus. Science **230**:1046–1048.
36. **Pintel, D., D. Dadachanji, C. R. Astell, and D. C. Ward.** 1983. The genome of minute virus of mice, an autonomous parvovirus, encodes two overlapping transcription units. Nucleic Acids Res. **11**:1019–1038.
37. **Pintel, D., M. J. Merchlinsky, and D. C. Ward.** 1984. Expression of minute virus of mice structural proteins in murine cell lines transformed by bovine papillomavirus-minute virus of mice plasmid chimera. J. Virol. **52**:320–327.
38. **Rhode, S. L., III.** 1978. Defective interfering particles of parvovirus H-1. J. Virol. **27**:347–356.
39. **Rhode, S. L., III.** 1978. Replication process of the parvovirus, H-1. X. Isolation of a mutant defective in replicative-form DNA replication. J. Virol. **25**:215–223.
40. **Rhode, S. L., III.** 1985. *trans*-Activation of parvovirus P_{38} promoter by the 76K noncapsid protein. J. Virol. **55**:886–889.
41. **Rhode, S. L., III.** 1985. Nucleotide sequence of the coat protein gene of canine parvovirus. J. Virol. **54**:630–633.
42. **Rhode, S. L., III, and P. R. Paradiso.** 1983. Parvovirus genome: nucleotide sequence of H-1 and mapping of its genes by hybrid-arrested translation. J. Virol. **45**:173–184.
43. **Ron, D., P. Tattersall, and J. Tal.** 1984. Formation of a host range mutant of the lymphotropic strain of minute virus of mice during persistent infection in mouse L cells. J. Virol. **52**:63–69.
44. **Sahli, R., G. K. McMaster, and B. Hirt.** 1985. DNA sequence comparison between two tissue-specific variants of the autonomous parvovirus, minute virus of mice. Nucleic Acids Res. **13**: 3617–3633.
45. **Sanger, F., S. Nicklen, and A. R. Caulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
46. **Shade, R. O., M. C. Blundell, S. F. Cotmore, P. Tattersall, and C. R. Astell.** 1986. Nucleotide sequence and genome organization of human parvovirus B19 isolated from the serum of a child during aplastic crisis. J. Virol. **58**:921–936.
47. **Siegl, G.** 1984. Canine parvovirus: origin and significance of a new pathogen, p. 259–295. *In* The parvoviruses. Plenum Publishing Corp., New York.
48. **Tattersall, P., and A. J. Shatkin.** 1977. Sequence homology between the structural polypeptides of minute virus of mice. J. Mol. Biol. **111**:375–394.
49. **Tratschin, J. D., G. K. McMaster, G. Kronauer, and G. Siegl.** 1982. Canine parvovirus: relationship to wild type and vaccine strains of feline panleukopenia virus and mink enteritis virus. J. Gen. Virol. **61**:33–41.