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Cloning and Sequence of DNA Encoding Structural Proteins of the Autonomous Parvovirus Feline Panleukopenia Virus

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Approximately 80% of the genome of feline panleukopenia virus was cloned into pBR322. This DNA included the transcription unit for the major viral mRNA species. The nucleotide sequence of the cloned portion of the genome was determined. Comparison of the feline panleukopenia virus sequence with the sequences of the parvoviruses minute virus of mice and H-1 revealed considerable homology between the three viruses on both the nucleic acid and protein levels. Based on this homology, a model for the generation of the two size classes of viral structural proteins (VP1 and VP2') is proposed.

Feline panleukopenia virus (FPV) is an autonomous parvovirus which infects cats. The virus attacks the lymph- and blood-forming tissues and the gastrointestinal mucosa, resulting in a drop in leukocyte count and enteritis (22). In newborn kittens, the virus may attack the developing cerebellum, resulting in neurological abnormalities. FPV is interesting not only because of the disease that it causes in cats but also because of two closely related variants which have emerged in recent times. In the late 1940s, outbreaks of severe enteritis occurred in ranch mink in Canada. The agent responsible for this was shown to be an autonomous parvovirus, mink enteritis virus (22). Similarly, in the late 1970s, widespread outbreaks of enteritis and myocarditis in dogs were shown to be caused by a parvovirus, canine parvovirus (6). FPV, canine parvovirus, and mink enteritis virus are all very closely related to each other both on the protein and nucleic acid levels, as shown by antigenic cross-reaction and restriction enzyme maps (14, 17, 25).

Parvoviruses are small, DNA-containing viruses which require actively dividing cells for replication. The genomes are linear single-stranded DNA molecules of ca. 5 kilobases (kb) in length. Virions generally contain three size classes of protein. A large 80,000- to 85,000-dalton protein (VP1) comprises ca. 10 to 15% of the viral protein. The remainder of the protein is a species of ca. 64,000 to 67,000 daltons (VP2'), part of which is converted to a 60,000- to 64,000-dalton species (VP2) by proteolytic cleavage (18). The amino acid sequences of the three proteins form a nested set. The entire sequence of the 60,000- to 64,000-dalton protein is contained within the 64,000- to 67,000-dalton protein, which in turn is contained within the 80,000- to 85,000-dalton protein (24).

In this communication, we report the cloning of ca. 80% of the genome of FPV into bacterial plasmids. This portion of the genome includes the sequences contained in the major mRNA in infected cells as demonstrated by single-stranded specific nuclease protection experiments. The nucleotide sequence of the cloned portion of the FPV genome is presented, and comparisons of the sequences of FPV, minute virus of mice (MVM), and H-1 are discussed. The sequence includes the gene for the capsid proteins of FPV and should be useful in the design of vaccines against FPV, canine parvovirus, and mink enteritis virus, based on recombinant DNA techniques, synthetic peptides, or other novel approaches.

MATERIALS AND METHODS

Cells, virus, bacterial strains, plasmids, and enzymes. FPV was grown in Crandell feline kidney cells which were maintained in medium F12 supplemented with 5% fetal calf serum. FPV used for cloning was isolated from infected Crandell feline kidney cells grown and provided by Biologics Corporation, Inc. (Omaha, Nebr.). Restriction fragments were cloned in pBR322 and grown in *Escherichia coli* K-12 strain HB101. Restriction fragments were also cloned into bacteriophage M13 strains mp2, mp7, and WJ22 and grown in *E. coli* strain JM101. Restriction endonucleases and other enzymes used in recombinant DNA work were purchased from Bethesda Research Laboratories (Rockville, Md.), New England BioLabs (Beverly, Mass.), Boehringer Mannheim Biochemicals (Indianapolis, Ind.), or P-L Biochemicals, Inc. (Milwaukee, Wis.). All enzymes were used as recommended by the manufacturers.

Isolation of FPV viral DNA and replicative-form DNA. FPV was purified from infected cells as described by Green et al. (8). Briefly, infected cells were disrupted by three cycles of freezing and thawing, followed by sonication. The lysate was treated with DNase, RNase, trypsin, and chymotrypsin and layered onto a sucrose-cesium chloride step gradient. After centrifugation, the virus was collected from the CsCl layer. DNA was extracted from the virus by treatment with 0.2% sodium dodecyl sulfate and 0.2% proteinase K, followed by phenol extraction and ethanol precipitation. Viral DNA was made double stranded for cloning by incubating at 30°C with *E. coli* DNA polymerase I (Klenow fragment) and 0.02 mM each deoxynucleoside triphosphate (5). FPV replicative-form DNA was isolated from nuclei of infected cells as outlined by McMaster et al. (13). Briefly, nuclei were disrupted with 1% sodium dodecyl sulfate, NaCl was added to 1 M, and the mixture was incubated at 4°C overnight. The mixture was centrifuged at 27,000 × *g*, and the supernatant containing low-molecular-weight DNA was ethanol precipitated, digested with 0.5 mg of proteinase K per ml, phenol

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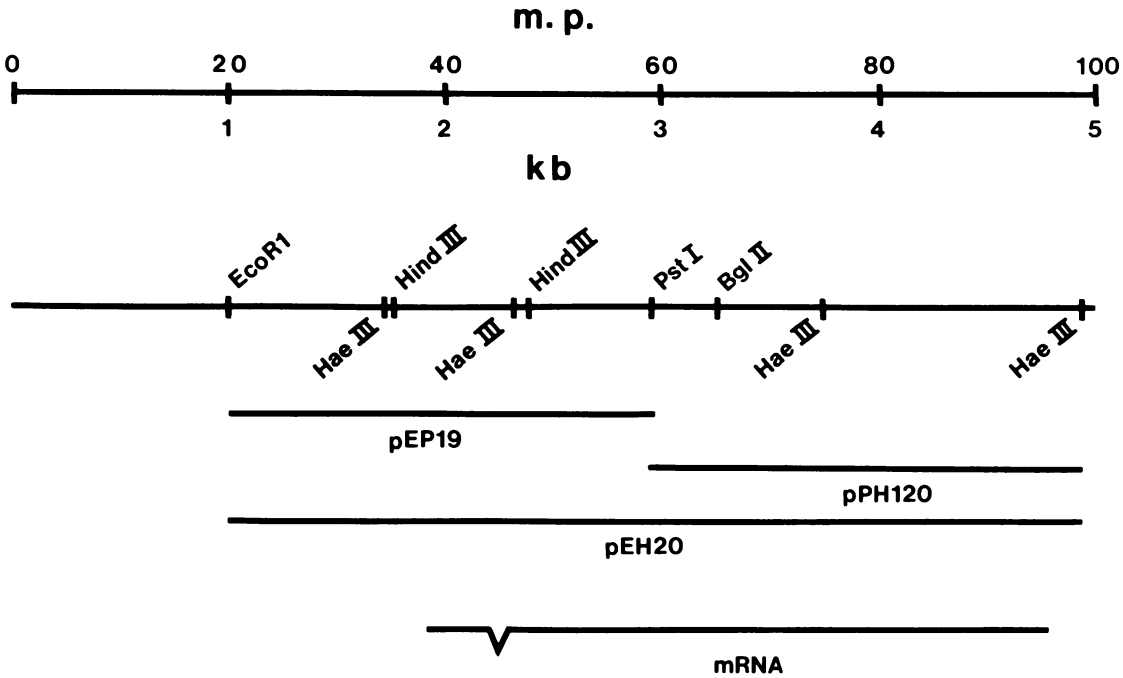


FIG. 1. Regions of the FPV genome cloned into plasmids. The scale of the genome is shown in map units (m.p.) and kilobases (kb) in the top line. The positions of several restriction sites used in cloning are shown in the second line. The portion of the genome contained in the plasmids pEP19, pPH120, and pEH20 are indicated below the restriction map. The DNA between the *Eco*RI site (m.p. 20) and the *Pst*I site (m.p. 58) was cloned between the *Eco*RI and *Pst*I sites of pBR322 to form pEP19. The DNA between the *Pst*I site (m.p. 58) and the *Hae*III site (m.p. 96) was cloned between the *Pst*I site and filled in *Eco*RI site of pBR322 to form pPH120. pEH20 was constructed by inserting the pEP19 and pPH120 inserts into the *Eco*RI site of pBR322. The portion of the genome contained in the major FPV mRNA species is also indicated.

extracted, and ethanol precipitated. The FPV replicative-form DNA was freed from contaminating cellular DNA by boiling and phenol extraction in 0.5 M NaCl. Because of its hairpin structure, a portion of the replicative-form DNA renatured immediately and was recovered in the aqueous phase as double-stranded DNA.

Single-stranded specific nuclease mapping of FPV-specific transcripts. Cytoplasmic RNA was isolated from uninfected or FPV-infected cells by suspending and lysing cells in 0.14 M NaCl–10 mM Tris (pH 8.2)–10 mM $MgCl_2$–0.2% Nonidet P-40. Nuclei were removed by centrifugation at 1,000 × *g*. The supernatant was extracted with phenol-chloroform (1:1), ethanol precipitated, incubated with proteinase K at 100 μg/ml, phenol extracted, and ethanol precipitated. Mapping of the regions of DNA which were transcribed into RNA was performed essentially as described by Berk and Sharp (4). Approximately 0.5 μg of the appropriate DNA was mixed with ca. 5 μg of total cytoplasmic RNA and ethanol precipitated. The precipitate was dissolved in 20 μl of 80% formamide–0.4 M NaCl–0.4 M PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)] (pH 6.4)–1 mM EDTA. The solution was sealed in a glass capillary, heated to 85°C for 5 min, and incubated at 45°C for hybridization. After hybridization overnight, the reaction mixture was diluted 50-fold into 50 mM NaCl–50 mM sodium acetate (pH 5.0)–1 mM zinc acetate–0.015 mg of denatured herring sperm DNA per ml. Mung bean nuclease was added to a final concentration of 143 U/ml, and the mixture was incubated at 30°C for 1 h. Ten micrograms of yeast tRNA was added as a carrier, and the reaction was stopped by the addition of EDTA to 2 mM, sodium acetate to 0.2 M, and 2.5 volumes of ethanol. The precipitated nucleic acids were redissolved in 10 mM Tris–1

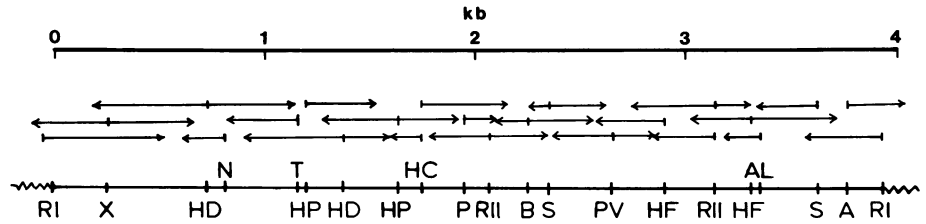


FIG. 2. Strategy for sequencing of pEH20. Restriction sites used in the determination of the sequence are indicated. All restriction fragments were labeled at the 5′ terminus. The direction and extent of the sequence obtained are indicated by arrows. Abbreviations used are as follows: RI, *Eco*RI; RII, *Eco*RII; X, *Xba*I; HD, *Hin*dIII; N, *Nco*I; T, *Taq*I; HP, *Hpa*I; AL, *Alu*I; HC, *Hin*cII; P, *Pst*I; PV, *Pvu*II; B, *Bgl*II; S, *Sau*3A; HF, *Hin*fI; and A, *Ava*II. The sequence from plasmid pBR322 is indicated by jagged lines at either end.

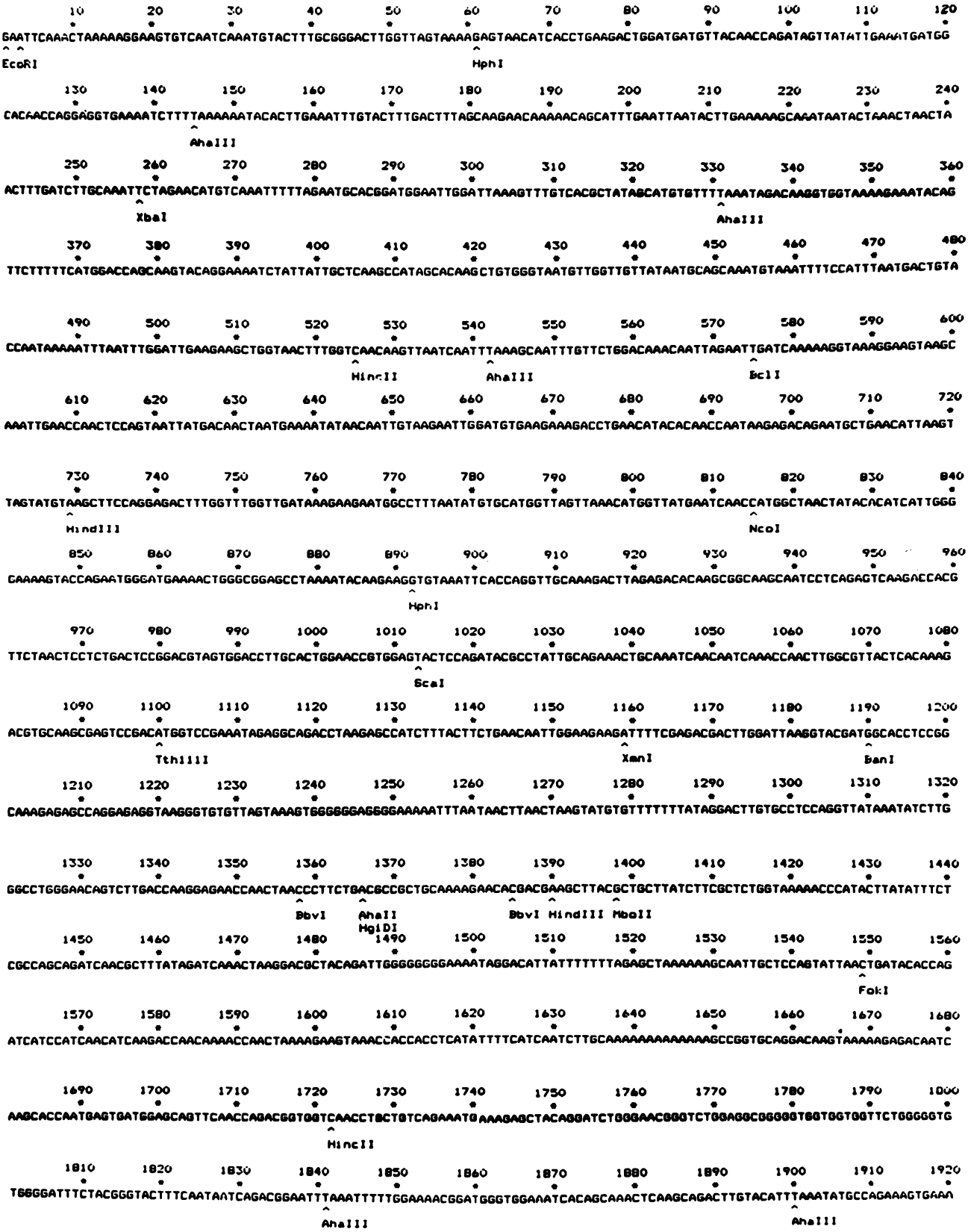
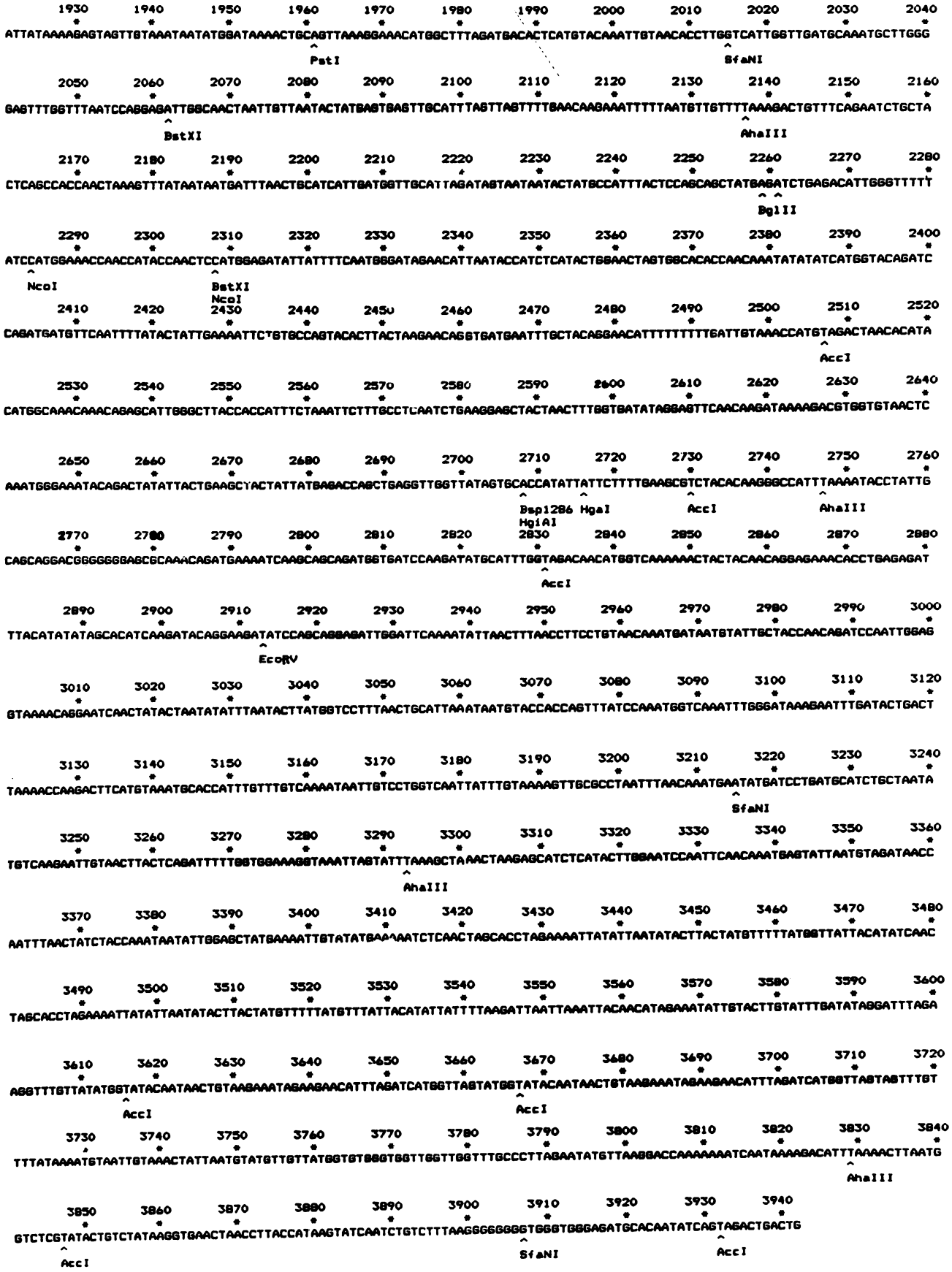


FIG. 3. Nucleotide sequence of the portion of the FPV genome contained in pEH20. The positions of a number of restriction enzyme cleavage sites are shown.


PstI
SfaNI
BstXI
AhaIII
BglII
NcoI
BstXI
NcoI
AccI
Bsp1286 HgaI
HgiAI
AccI
AhaIII
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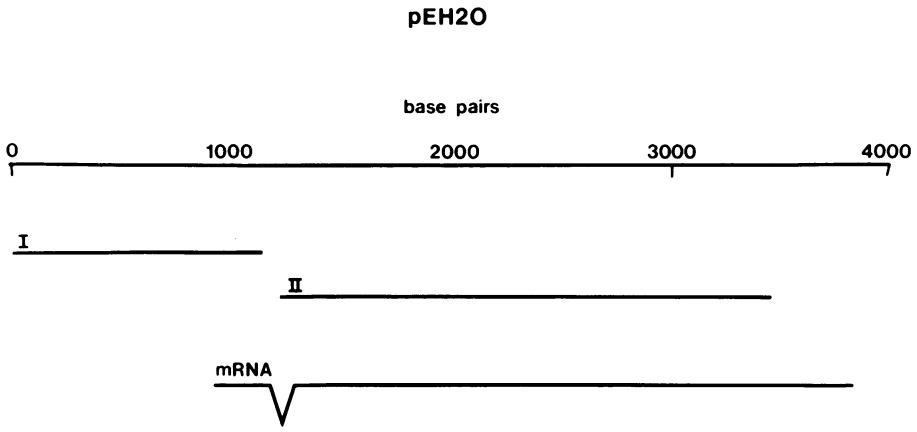

FIG. 4. Translational reading frames. The positions of the two long open translational reading frames in the sequence of pEH20 are indicated along with the portions of the sequence contained in the major FPV-specific mRNA species.

mM EDTA (pH 7.4), fractionated on an alkaline agarose gel, blotted onto diazobenzyloxymethyl-paper, and annealed to appropriate ^{32}P-labeled, nick-translated DNA probes (26).

RESULTS

Cloning of FPV. The 3′ end of DNA isolated from parvovirus virions contains inverted repeat sequences which form hairpin-like structures (1). Because of this structure, parvoviral DNA can function as a primer-template molecule for DNA polymerase. DNA from CsCl-purified FPV was converted to double-stranded DNA with *E. coli* DNA polymerase (5, 15) and used to clone the fragment between the *Eco*RI site at map position (m.p.) 20 and the *Pst*I site at m.p. 58 (plasmid pEP19, Fig. 1). Synthesis of full-length, double-stranded DNA with polymerase and single-stranded viral DNA proved difficult. DNA between the *Pst*I site at m.p. 58 and the *Hae*III site at m.p. 96 was cloned from double-stranded, replicative-form DNA isolated from the nuclei of infected cells (plasmid pPH120, Fig. 1). A plasmid containing the entire FPV sequence between the *Eco*RI site at m.p. 20 and the *Hae*III site at m.p. 96 was constructed with the FPV inserts from pEP19 and pPH120. To have a convenient source of single-stranded DNA for single-stranded specific nuclease mapping of FPV mRNA, several fragments were cloned into appropriate bacteriophage M13 derivatives. The *Eco*RI-*Pst*I fragment from pEP19 was cloned into M13mp7. The *Hin*dIII fragment between m.p. 34 and m.p. 48 was cloned into M13WJ22. The FPV fragment from pEH20 was cloned into M13mp2.

Sequence of pEH20. The sequence of the FPV portion of pEH20 was determined by the method of Maxam and Gilbert (11) according to the strategy shown in Fig. 2. The entire sequence of 3,948 base pairs (bp) is shown in Fig. 3. The sequence shown is that of the C strand which is complementary to the V strand packaged in the virion. The C strand contains the sequences present in the mRNAs. Restriction endonuclease cleavage sites predicted from the sequence generally agree with the published maps for FPV and mink enteritis virus (14, 25). The numbering of the pEH20 sequence begins at the *Eco*RI site at m.p. 20, ca. 1 kb from the left end of the FPV genome. The nucleotide sequences of H-1 and MVM are numbered from the left ends of the genomes and contain *Eco*RI sites at nucleotides 1087 and 1084, respectively (2, 21). If the three sequences are aligned at their *Eco*RI sites, then there is considerable base sequence homology among the three viruses. Therefore, comparison of the FPV sequence with the H-1 and MVM sequences is facilitated by the addition of ca. 1,100 to the number in the pEH20 sequence. The nucleotide sequence of pEH20 contains two long open translational reading frames (Fig. 4). As in MVM and H-1, one of these reading frames (frame I) includes most of the left half, and the other (frame II) includes most of the right half of the genome. Frame I includes the *Eco*RI site and terminates at the TAA codon at base 1181. Since the DNA to the left of the *Eco*RI site at ca. m.p. 20 was not cloned, the precise position of the beginning of frame I on the FPV genome is unknown. Frame II begins at base 1269 and terminates with a TAA codon at base 3443. Homology among the viruses is greater in frame I than in frame II (Table 1). FPV is about equally homologous with MVM and H-1, which are substantially more homologous to each other than to FPV in both reading frames. Electron microscopic studies on heteroduplex DNA from a number of parvoviruses have shown that the DNA sequences in the left half of the genome are highly conserved among H-1, MVM, LuIII, and Kilham rat virus. The right half of the genome is much less conserved (3). These results are consistent with the direct sequence comparison of FPV, H-1, and MVM.

The long reading frame from the left-hand portion of the genome of MVM codes for a nonstructural protein of unknown function. This protein reacts with antisera from convalescent animals of several species infected with various parvoviruses (7). The amino acid sequences of FPV and MVM (frame I) differ in only 89 of 397 positions. Fifty-one of these differences are in the carboxy-terminal 104 amino acids. For comparison, H-1 and MVM differ in only 35 of 397

TABLE 1. Nucleic acid and protein sequence homology among parvoviruses[a]

Virus pair	% Homology for:			
	Frame I		Frame II	
	Nucleotide	Amino acid	Nucleotide	Amino acid
FPV–MVM	73	78	55	51
FPV–H-1	72	78	55	52
MVM–H-1	88	91	72	71

[a] The homology between pairs of viruses for the two long open reading frames (frame I and frame II) is shown for both nucleotide and predicted amino acid sequences.

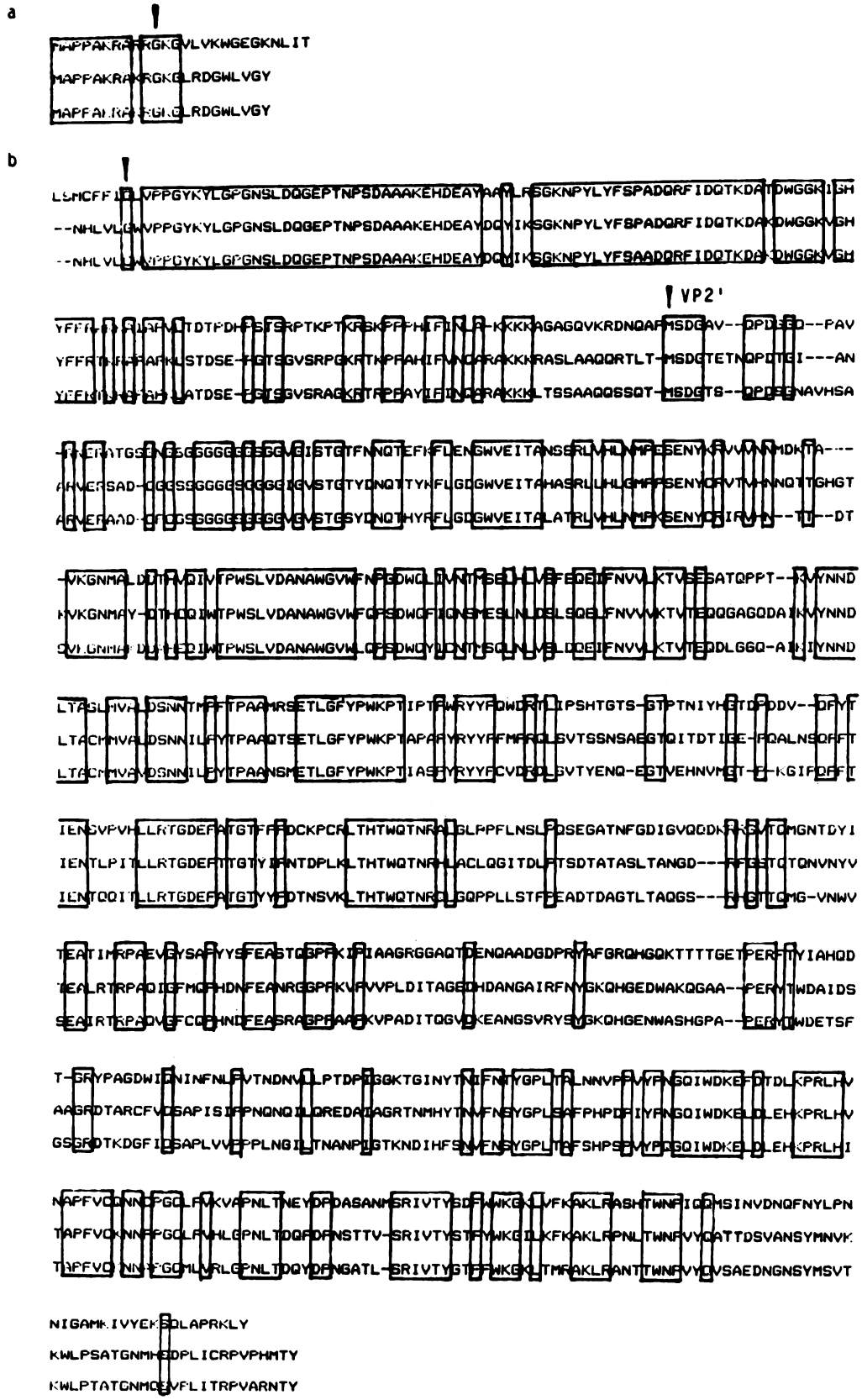

FIG. 5. Comparison of amino acid sequences of the capsid proteins of FPV, H-1, and MVM. The amino acid sequences from the short reading frames postulated to code for the amino termini of VP1 are shown in (a). The sequences of FPV, H-1, and MVM are the top, middle, and bottom lines, respectively. The position of the putative 5′-splice junction at nucleotide 1218 is indicated by a vertical arrow. The amino acid sequences from the long open translational reading frames are shown in (b). The position of the putative 3′-splice junction at nucleotide 1291 is indicated by a vertical arrow. Spaces were introduced in the sequences to maximize homology. Regions of identical sequence in the three proteins are outlined in boxes. The postulated amino termini for VP2′ are also indicated.

positions, and 23 of these differences are clustered in the carboxy-terminal 104 amino acids.

The right half of the genome in MVM and H-1 has been shown to code for the structural proteins of the virion (7, 16, 21). Figure 5 compares the amino acid sequence predicted from frame II with H-1 and MVM. Approximately the first 100 amino acids near the amino terminus are highly conserved among the three viruses. In general, regions which are homologous between FPV and H-1 are also homologous between FPV and MVM. This suggests that these regions may have some critical functional significance in the structural proteins of parvoviruses. It is interesting that despite extensive amino acid homology among parvoviruses, there is little immunological cross-reactivity among them (23).

Single-stranded specific nuclease mapping of FPV transcripts. The mRNA species produced in MVM infection have been mapped in detail (19). To determine whether the major message was similar in FPV, single-stranded specific nuclease mapping experiments were performed. When cytoplasmic RNA from FPV-infected cells was hybridized to the sequences contained in pEH20, fragments of 270 and 2,500 bp were protected (data not shown). When RNA was hybridized to the DNA contained in pEP19, fragments of 270 and 660 bp were protected (Fig. 6, lanes 5 and 6). When the DNA between the *Hin*dIII sites at m.p. 34 and m.p. 48 (nucleotides 729 through 1390) was used, a fragment of 270 bp was protected (Fig. 6, lanes 1 and 2). These results are consistent with a major mRNA species composed of a 270-bp exon mapping between the *Hin*dIII sites which is spliced to an uninterrupted stretch of 2,500 bp complementary to the 5′ half of the genome. A similar result is observed for the major mRNA in MVM- or H-1-infected cells. No attempt was made to map the minor viral mRNA species.

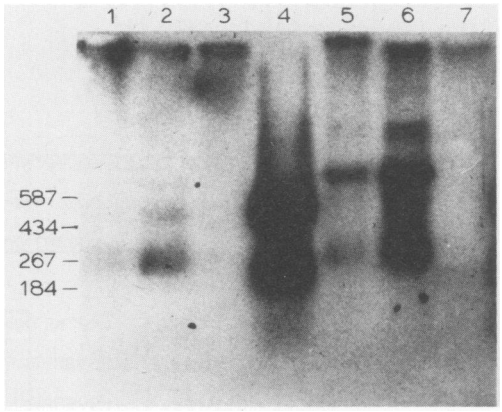

FIG. 6. Mapping of RNA sequences by single-stranded specific nuclease protection experiments. Cytoplasmic RNA from uninfected or FPV-infected CFK cells was hybridized with single-stranded DNA from M13 phage containing various FPV inserts. The hybrids were digested with mung bean nuclease, fractionated on alkaline agarose gels, blotted onto diazobenzyloxymethyl-paper and probed with ^{32}P-labeled FPV DNA. An autoradiogram of a blot is shown. M13 phage DNA (0.5 μg) containing the *Hin*dIII fragment (m.p. 34 to m.p. 48) was hybridized with 1 μg of infected cell RNA (lane 1), 5 μg of infected cell RNA (lane 2), and 5 μg of uninfected cell RNA (lane 3). Lane 4 is a *Hae*III digest of pBR322 DNA used as molecular weight markers. M13 phage DNA (0.5 μg) containing the EP19 insert (m.p. 20 to m.p. 58) was hybridized to 1 μg of infected cell RNA (lane 5), 5 μg of infected cell RNA (lane 6), and 5 μg of uninfected cell RNA (lane 7). The positions of four molecular weight markers are indicated in the left margin.

DISCUSSION

Transcriptional control sites. Transcription of the major virus-specific mRNA in MVM and H-1 begins at a promoter at ca. m.p. 39. An intervening sequence mapping at ca. m.p. 46 is removed from the primary transcript to produce the mature mRNA. Consistent with this, the nucleotide sequence of MVM and H-1 contains the sequence TATAAAT at m.p. 39. This sequence, the so-called TATA box, is characteristic of eucaryotic promoters and is situated ca. 30 bp upstream from the transcriptional initiation site. This region in MVM has been shown to function as a promoter in the in vitro transcription system developed by Manley, with transcription initiating ca. 30 bp downstream from the TATA box (19). Figure 7 shows the DNA sequences of FPV, H-1, and MVM aligned beginning at this sequence. In FPV the sequence (beginning at nucleotide 893) is changed to TGTAAAT, an unusual but not unprecedented change. A similar sequence, TAAAATA (not conserved in H-1 or MVM), occurs 15 bases upstream at nucleotide 878. Either or both of these sequences might function in determining the position of the transcription initiation site in FPV. There is an AATAAA at nucleotide 3816 which probably marks the end of the mature transcript in FPV. This sequence is characteristic of polyadenylation sites for eucaryotic mRNAs and is also found in multiple copies at approximately the same position in both the H-1 and MVM sequences. A transcript beginning around nucleotide 920 and ending near nucleotide 3816 would contain most if not all of the sequences coding for the viral structural proteins VP1 and VP2′.

Upstream from the postulated polyadenylation site in FPV are two sets of direct repeat sequences. The first set, a 60-base repeat (nucleotides 3417 to 3476 and 3477 to 3537), overlaps the long rightward open reading frame. There is a 1-base mismatch between the repeats (base 3565 is a G and base 3525 is a T). The other set is a 51-base repeat (nucleotides 3611 to 3661 and 3662 to 3712). The significance of these direct repeats is uncertain, but it is interesting to note that the H-1 sequence contains a set of 55-base repeats in a similar location (20).

Coding sequences for capsid proteins. The similarity in size and relative abundance of the capsid proteins (VP1 and VP2′) among parvoviruses makes it likely that the transcription and translation signals will be conserved. Initiation of translation at the ATG at nucleotide 1689 would result in a protein of 584 amino acids with a calculated molecular weight of 64,661, based on amino acid sequence. This agrees quite well with the molecular weight of VP2′ (64,000 to 67,000) as determined by gel electrophoresis (18). Furthermore, this ATG codon and the three codons immediately following it are conserved among FPV, H-1, and MVM (Fig. 5b). This codon has recently been found to initiate synthesis of VP2′ in H-1 (16).

The initiation of translation of VP1 could begin at the ATG codon at position 1275. This would yield a protein of 722 amino acids with a calculated molecular weight of 79,845, which agrees reasonably well with estimates of 82,000 to 83,000 as measured by gel electrophoresis. However, this initiation codon is not found in H-1 and MVM. Alternatively, translation of VP1 could begin at the ATG at position 1188. This codon begins an open reading frame whose amino acid sequence is highly conserved among FPV, MVM, and H-1 for 13 amino acids and then terminates shortly thereafter (Fig. 5a). Comparison of the sequences of the three viruses (Fig. 7) shows that the sequence AGGTAAG at position 1217

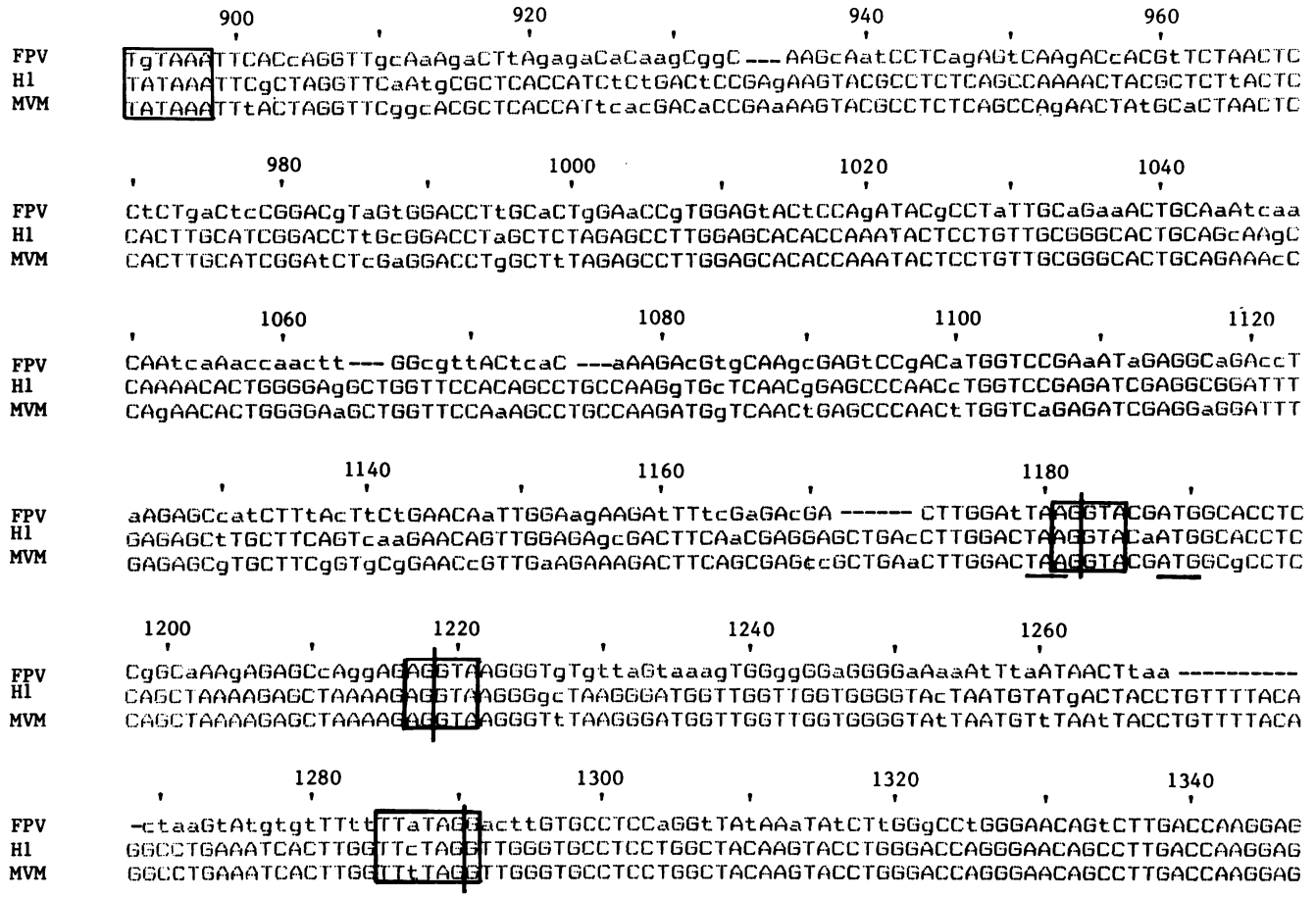

FIG. 7. Comparison of nucleotide sequences of FPV, H-1, and MVM in the mRNA promoter and splicing regions of the genomes. The sequences are aligned at the TATAAA boxes. Spaces are added to the FPV sequence to maximize homology. The numbering system is from the pEH20 sequence. The H-1 sequence shown begins at nucleotide 1979 (12). The MVM sequence shown begins at nucleotide 1976 (14). Nucleotides which are identical in at least two of the three sequences are shown as capital letters. Nucleotides which differ from the consensus are shown in lowercase. Conserved sequences which may function as 5′-splice junctions are shown in boxes at nucleotides 1181 and 1217. Conserved sequences which may function as a 3′-splice junction are shown in a box at nucleotide 1285. The termination codon for the leftward open reading frame is underlined at nucleotide 1179. The conserved ATG codon postulated to initiate VP1 is underlined at nucleotide 1188.

in FPV is conserved in the three viruses. This sequence agrees well with the consensus sequence for 5′-splice junctions (C/A)AG ‖ GT(A/G)AGT (12). This putative splice junction is very near the end of the amino acid homology in this reading frame between FPV and the other two viruses. Further downstream at position 1285 is the sequence TTATAGG, which conforms well to the consensus sequence for 3′-splice junctions (T/C)nN(C/T)AG ‖ G (12). The sequence is also largely conserved in MVM and H-1. It is interesting to note that this postulated 3′-splice junction is very close to the position in which the amino acid homology between the long right-hand open reading frames of the three viruses begins. If these are the splice junctions used, then a short stretch of 10 codons (beginning with the ATG at position 1188 and ending with the G at position 1218) would be fused at the G at position 1291 to create an open reading frame of 727 codons. This open reading frame would code for a protein with a calculated molecular weight of 80,366. This agrees reasonably well with the observed molecular weight of VP1.

For the initiation of the smaller of the two virion proteins (VP2′) to occur at the ATG at position 1689, it might be necessary to remove the proposed ATG for VP1 at position 1188. The presence of a second donor consensus sequence just upstream from this ATG suggests a mechanism for this removal. The sequence AAGGTA at position 1180 is conserved among the three parvoviruses and agrees well with the 5′-splice junction consensus sequence. If this splice junction at 1180 were joined to the 3′-splice junction at 1291, the ATG at 1188 would be removed, allowing the use of the next ATG at position 1689 for translation initiation of VP2′. If transcription initiates ca. 30 bp downstream from the TGTAAAT sequence at position 893 and the splice event joining nucleotide 1180 to 1291 is used, then the resulting mRNA would consist of a 5′ exon of ca. 260 bp spliced to a 3′ exon of ca. 2.5 kb. This is consistent with the single-stranded specific nuclease experiments described above. If, on the other hand, the 5′-splice junction at 1217 is joined to the 3′-splice junction at 1291, then translation would begin at the ATG at position 1188 and VP1 would be synthesized. This mRNA would consist of a 5′ exon of ca. 290 bp spliced to a 2.5-kb exon. MVM and H-1 (and probably FPV) also produce doubly spliced RNA which is transcribed from a promoter at ca. m.p. 4. Introns of ca. 1.5 kb, located between m.p. 8 and m.p. 38, and 100 bases located around m.p. 45 are removed from this species. Either one of the

splicing schemes described above could be used to remove the intron at m.p. 45 from this RNA as well.

The translational scheme outlined above would generate proteins of reasonable molecular weights and involves features highly conserved among the three viruses. However, translational initiation of the coat proteins would probably not occur at the first AUG in the FPV mRNAs. If transcription begins ca. 30 bp downstream from the TGTAAAT sequence (at nucleotide 993), then the first ATG in the mRNA would be at nucleotide 1100. This reading frame is terminated 15 codons downstream by a TGA at position 1142. This ATG is not present in H-1 or MVM. Although translation initiates at the first AUG in many eucaryotic mRNAs, numerous exceptions have been described. Recent results suggest that initiation at a downstream AUG can occur efficiently, provided that the upstream AUG is followed by an in-frame termination codon (9, 10).

FPV has many features in common with the more thoroughly studied parvoviruses, H-1 and MVM. The major mRNA species maps in the same region of the genome in all three viruses. Comparison of the DNA sequences of FPV, H-1, and MVM in the putative promoter and splicing regions (Fig. 7) allowed us to propose a model for generating mRNAs coding for either VP1 or VP2′. This model proposes an alternate 5′-splice junction joining to a common 3′-splice junction. The proposed splice junctions conform reasonably well to consensus sequences, and all are conserved among the three parvoviruses.

Although the region of the FPV genome between m.p. 0 and ca. m.p. 20 was not cloned, it is evident that the protein coded for by the long leftward open reading frame exhibits extensive homology to that coded by H-1 and MVM. This is not surprising, since antisera from animals infected with various parvoviruses are able to immunoprecipitate this protein from in vitro translation products of MVM mRNA (7). The portion of the genome which codes for the structural proteins of FPV (the long rightward open reading frame) exhibits less homology with H-1 and MVM. Nevertheless, comparison of the predicted protein sequences for the viruses shows that there is substantial conservation of sequences between the viral proteins. During maturation of parvoviruses, a portion of VP2′ is converted to VP2 by a proteolytic cleavage which removes ca. 30 amino acids from the amino terminus of VP2′. If VP2′ initiates with the ATG at position 1689, then the cleavage point would be in an unusual sequence composed mainly of glycine residues with a few serines interspersed. This glycine-serine-rich region is conserved among all three parvoviruses.

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