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LETTER TO THE EDITOR

Nucleotide Sequence of the Inter-Structural Gene Region of Feline Infectious Peritonitis Virus

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Abstract. The sequence of the region located between the S and M glycoprotein genes of the 79-1146 strain of feline infectious peritonitis virus (FIPV) is presented. The inter-structural gene region encodes 3 open reading frames (ORFs), termed ORFs 3a, 3b and 4, with nucleotide sequences conforming to the minimum conserved transcription signal upstream of each. An additional ORF, 3x, partially overlaps the 3′ end of ORF 3a. The FIPV interstructural gene region is identical in length when compared to the Insavc-1 strain of canine coronavirus (CCV) but differs from various strains of transmissible gastroenteritis virus (TGEV) by the presence of deletions and insertions. The sizes of ORF 3a and 4 are conserved in FIPV, TGEV and CCV. However, as with CCV, the FIPV ORF 3b is truncated in comparison with TGEV.

Key words: feline infectious peritonitis virus, open reading frames 3a, 3b and 4, comparative analysis

Feline infectious peritonitis is a disease characterized by immunopathology and caused by a coronavirus. In FIPV-infected cells, 7 viral mRNAs have been detected (1). Two of these originate from a region of the FIPV genome lying between the genes encoding S and M. This inter-structural gene region has been examined in the related coronaviruses transmissible gastroenteritis virus (TGEV) and canine coronavirus (CCV) (2–6). The arrangement of the open reading frames (ORFs) in the inter-structural gene region has been described for the FIPV genome (1,7), but detailed sequence has not been presented. Here, we report on the sequence of this part of the FIPV genome.

We screened an FIPV 79-1146 cDNA library with oligonucleotide probes derived from the published S sequence (8) and isolated clones containing the inter-structural gene region. The sequence of one of these cDNAs (GenBank accession number AF033000) was analyzed in detail.

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The overall organization of the FIPV inter-structural gene region is similar to that of CCV and TGEV. Three ORFs encoding polypeptides of 71, 40 and 82 residues are present in the FIPV sequence; these have been designated ORF 3a, 3b and 4, respectively, in CCV and TGEV (ORF 4 is also known as the small membrane gene because it encodes a polypeptide that is similar in sequence to an infectious bronchitis virus membrane protein). An additional ORF of 71 residues partially overlaps the 3′ end of FIPV ORF 3a. This ORF is present in CCV but absent from TGEV, and has been called ORF 3x (6). Upstream of the FIPV ORF 3a and ORF 4 is the nucleotide sequence CTAAAC that is the minimum conserved transcription signal found in other FIPV, TGEV and CCV genes (6,9). A related sequence, CTAAAT, is present upstream of FIPV ORF 3b. For these ORFs, the distance between the transcription signal and the start of translation is conserved in the 3 coronaviruses.

The inter-structural gene regions of FIPV and the Insavc-1 strain of CCV are identical in size. The

sequence identity of the two regions is 89.7% at the nucleotide level.

A 52 base deletion, located 59 bases upstream of ORF 3a, and a 63 base insertion, starting 153 bases upstream of ORF 3b, are present in the FIPV inter-structural gene region relative to the Purdue strain of TGEV. Different strains of TGEV also show variation in these same regions, emphasizing the sequence flexibility in this part of the coronavirus genome. The sequence identity between FIPV 79-1146 and the Purdue strain of TGEV in the inter-structural gene region is 90.7%.

The products of FIPV ORF 3a and ORF 4 are identical in length to the corresponding polypeptides of CCV and TGEV (Purdue strain), with amino acid similarities of 91.5% and 81.7%, respectively, between FIPV and CCV, and 90.1% and 75.6% between FIPV and TGEV. In contrast, an amber codon limits FIPV ORF 3b to only 40 residues while ORF 3b of the Purdue strain of TGEV extends 244 amino acids (3). Although the FIPV ORF 3b is shorter, the region distal to the amber codon is similar in nucleotide sequence and identical in length to the remaining portion of the TGEV ORF 3b sequence. This is consistent with the idea that a base substitution has created a premature stop codon in the FIPV ORF 3b coding region. For CCV, ORF 3b is also limited to 31 residues.

Differences in expression and primary sequence of ORF 3b occur in various TGEV strains, and ORF 3b is truncated in FIPV and CCV. This indicates that ORF 3b is not absolutely required for virus growth. Sequence determination of virus passaged in cats will help to answer questions about the requirement for ORF 3b expression in the virus life cycle (7).

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