Analysis of the genetic information of a DNA segment of a new virus from silkworm

Brief Report

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Summary. In 1983, a parvo-like virus (Yamanashi isolate) was newly isolated from silkworm. However, unlike parvovirus, two DNA molecules (VD1 and 2) were always extracted from purified virions. To investigate the structure and organization of the virus genomes, we determined the complete nucleotide sequence of VD2. The sequence consisted of 6031 nucleotides (nts) and contained a large open reading frame (ORF1) with 3513 nts. A smaller open reading frame (ORF2) with 702 nts was found in the complementary sequence. Computer analysis revealed that both ORFs did not code for the major structural proteins (VP1, 2, 3, and 4). These results suggest that VD2 has not enough information to produce progeny virions by itself. Further, the structural importance of the terminal sequence (CTS) common to both VD1 and VD2 was also predicted by a computer analysis.

* The viruses containing a small, single-stranded linear DNA genome are classified into the family Paroviridae [18]. Densovirus (DNV) is the invertebrate parovirus with the ability of autonomous replication. In these 25 years, more than 20 species of densoviruses have been isolated from many kinds of insect [20]. Only a few DNVs have been investigated in some detail and the DNV isolated from Bombyx mori (Ina isolate) which is an agent causing flacherie disease of silkworm is one of the most studied DNVs. The Bm DNV (Ina isolate) encapsidates both “+” and “−” strands (about 5000 nucleotides length) in separate capsids in about equal amounts [2, 15]. This results in the yield of a double-stranded DNA molecule by the extraction under condition of appro-
appropriate ionic strength. The Bm DNV-DNA also has inverted terminal repeats of 225 nucleotides. These characteristics were very similar to those of other parvoviruses, especially to AAV. However, unlike AAV, the small internal palindrome which forms a T-shaped conformation could not be observed in the terminal repeats. End-label analysis demonstrated that the palindromic sequences at both termini can exist in either of two orientations (flip or flop) in virion DNA with different frequencies. These results suggested that the hairpin transfer model for AAV replication could be adopted to explain the DNV replication with some modifications [2].

About ten years ago, other agents of flacherie disease of silkworm which showed no serological relationship with the Bm DNV (Ina isolate) were isolated independently from different areas in Japan [13, 7]. These two isolates were serologically indistinguishable though they were designated as “Yamanashi isolate” and “Saku isolate” in relation to the district they were obtained from. The characteristic traits of these isolates were quite similar to those of densovirus [11, 13], and these were tentatively designated as Bm DNV-2 while “Ina isolate” as Bm DNV-1 [21]. However, unlike other DNVs, the Bm DNV-2 (Yamanashi isolate) encapsidates two sets of complementary DNA strands, probably in separate capsids [3]. Therefore, extraction of DNA yields two double-stranded DNA molecules with slightly different electro-mobilities (VD1: 6.6 kb; VD2: 6.1 kb). Southern hybridization experiments indicated that the nucleotide sequence of VD2 was quite different from that of VD1, though the following partial sequencing analysis revealed that they shared a common terminal sequence of 53 nucleotides [3]. However, the terminal palindrome common to all parvoviruses could not be observed at the termini of VD1 and 2 [3], suggesting that the replication mechanism of Bm DNV-2 was different from that of parvoviruses. The genome organization and the genetic information on the viral DNAs are still obscure, making it difficult to conclude whether the Bm DNV-2 is the mixture of two parvo-like viruses or a virus with a multipartite genome. In this report, we determined the nucleotide sequence of VD2 and investigated the genetic information in the viral DNA segment.

To determine the nucleotide sequence of VD2, serial deletion mutants of the clone (pV1171) containing a complete nucleotide sequence of VD2 [3] were made by endonuclease III followed by a mung bean nuclease according to the method described by Henikoff [9]. Sequencing was performed by dideoxy chain termination method [16] using the T7 DNA polymerase (US Biochemical Corp., Cleveland, OH) and [α-35S]dATP (DuPont, NEN Research Products, Boston, MA). A nucleotide analogue deazaguanosine triphosphate (7-deaza GTP) was used in a reaction with Taq DNA polymerase to resolve the regions high in G+C content. These products were purchased from U.S. Biochemical Co. Oligonucleotides synthesized with an Applied Biosystems DNA synthesizer (Applied Biosystems, Inc., CA) were also used as primers for sequencing. Analysis of the sequence data were aided by SDC-GENETYX program (Software Development Co., Ltd) and DNASIS DNA sequence analysis system (Hitachi Software Engineering America, Ltd.).