Nucleotide Sequence Analysis of the Penaeid Rod-shaped DNA Virus: Taxonomic Implications

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Introduction

The penaeid rod-shaped DNA virus (PRDV), newly isolated from the kuruma shrimp, *Penaeus japonicus*, in Japan possesses a non-segmented, double-stranded DNA genome (1). In Southern hybridization experiments, restriction fragments of PRDV DNA did not hybridize with DNA genome of *Bombyx mori* nucleopolyhedrovirus (BmNPV), a member of the Baculoviridae. On the contrary, restriction fragments of BmNPV DNA did not hybridize with PRDV DNA. These results suggested that the DNA genome of PRDV did not contain any sequences highly homologous to those of baculoviruses.

Nucleotide sequence analysis of PRDV DNA

To reveal the position of PRDV in the virus taxonomy, nucleotide sequences of several DNA fragments of PRDV genome were determined and analyzed. More than a dozen of open reading frames (ORFs) were identified. Most of them did not show any significant homologies to gene products of DNA viruses in the SWISS-PROT protein database. However, three ORFs have been found to have curious characteristics as described below.

Two large ORFs potentially encoding a 44-kDa polypeptide and a 43-kDa amino-terminal portion of polypeptide were found in the opposite direction each other in the 2.9-kbp *Hin*dIII fragment. Analysis of the predicted amino acid sequence of the 44-kDa ORF revealed an aspartate-rich potential EF-hand calcium-binding domain. Although this aspartate-rich region showed weak similarities to the DNA polymerases of *Autographa californica* NPV (AcMNPV) (2) and BmNPV (3), any conserved motifs for DNA polymerases were not found in its amino acid sequence. The predicted amino acid sequence of the partial 43-kDa ORF contained a proline-rich region showing relatively high similarities to the EBNA-2 of Epstein-Barr virus (4) and two proteins of AcMNPV, the hypothetical 24.1-kDa protein and 61-kDa protein (5). No conserved motifs were found in this ORF.

An ORF found in the 4.5-kbp partially digested *Eco*RI fragment potentially encoding a 34-kDa carboxyl-terminal portion of polypeptide contained a collagen-like glycine and proline-rich repeat. There are no double-stranded DNA viruses except Herpesvirus saimiri possessing the gene encoding collagen-like protein (6). However, differences in the shape of virion as well as the host range between PRDV and herpesviruses indicate that a separate evolutionary origin for each collagen-like protein should be considered.

Relationship between PRDV and other novel shrimp viruses in Asia

Not only in Japan, PRDV-like novel shrimp DNA viruses appeared in Asian countries and have been causing severe damages in shrimp production since 1992. Reports from these countries indicates the white spot syndrome associated virus in Taiwan (7, 8), the systemic ectodermal and mesodermal baculovirus in Thailand (9) and a member of subgroup C of baculovirus in China (10) have almost the same virion structures and restriction profiles of genome DNA with those of PRDV.

According to the nucleotide sequence of PRDV DNA, three PRDV-specific PCR primer pairs were synthesized and PCR was performed using PRDV DNA and the DNA genome of the shrimp virus isolated in China as templates. For any of three primer pairs, DNA fragments with the same length were amplified from both template DNAs, strongly suggested PRDV and the Chinese isolate are the same virus, or at least closely related viruses.

Conclusion

Up to now, PRDV can not be classified into any virus groups but is one of the unassigned rod-shaped double-stranded DNA viruses of invertebrates such as *Oryctes rhinoceros* virus and *Heliothis zea* virus 1 (11), which were previously classified in the family *Baculoviridae*. Further sequence analysis as well as comparative studies between PRDV and other double-stranded DNA viruses isolated from not only penaeid shrimps but also other crustaceans will provide reliable genetic evidence to determine or establish the virus family in which PRDV should belong. Establishment of cell lines supporting shrimp virus replication in vitro will promote the progress in these studies. Accumulation of genetic information on the shrimp viruses will also enable us to utilize the information to develop novel methods for protecting shrimps from virus diseases.

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