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Sequencing of a 5.5-kb DNA Fragment and Identification of a Gene Coding for a Subunit of the Helicase/Primase Complex of Avian Laryngotracheitis virus (ILTV)

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Abstract. The nucleotide sequence of a 5,520-bp *Eco*RI restriction fragment of avian infectious laryngotracheitis (ILTV) DNA was reported and submitted to GeneBank with an accession number of AF001078. Computer prediction revealed one large potential open reading frame (ORF) with sequence similar to one subunit of the DNA helicase-primase complex of α-herpesviruses. The DNA helicase/primase complex of HSV-1 consists of three sub-units with molecular weights of 12,000, 97,000 and 70,000, encoded by genes UL52, UL5 and UL8, respectively. This enzyme complex is essential for herpesvirus DNA replication. The UL52 and UL5-equivalent genes of ILTV have been reported previously (Fuchs, W. and Mettenleiter, T.C., J Gen Virol, 1996, 77: 2221–2229; Johnson, M.A. et. al., Arch Virol, 1995, 14: 623–634). Amino acid sequence comparison and homology search revealed that this ORF shares sequence similarity to the UL8-equivalent gene of α-herpesviruses, that is, the ORF 52 of vericura-zoster virus (VZV), the ORF 54 of equine herpesvirus type-1 (EHV-1), as well as the equivalent gene of bovine herpesvirus type 1(BHV-1) and canine herpesvirus (Vlcek, C. et al., Virology, 1995, 210: 100–108; Remond, M. et al., J Gen Virol, 1996, 77: 37–48).

Key words: DNA helicase, primase complex, herpesvirus DNA, UL8, ILTV, DNA replicase

Introduction

Avian infectious laryngotracheitis virus (ILTV), or Gallid Herpesvirus, is a member of the α-subfamily of herpesviruses (1). The phylogenetics and molecular-evolution of ILTV have been addressed by comparing gene sequences of ILTV with that of other α-herpesvirus (2–4). The low sequence identity with other α-herpesviruses and the large number of conservative substitutions in ILTV genes suggest a considerably divergent history among this herpes family. Thus this warrants further investigation of the ILTV genome organization and sequence.

The sequences of twelve genes, equivalent to that coding for thymidine kinase (5,6), serine-rich protein (7), gB (2,8), P40 (9), helicase (4), uracil-DNA glycosylase (10), gD (3,11), gK (4), gC (12), ICP4 (13), ICP27 and ICP8 (14) of herpes simplex virus as well as the 32-kDa immunogenic protein (15), have been described for ILTV.

It is of significant importance to better understand the biology of ILTV in the hopes of developing attenuated recombinant vaccines that do not induce latency in immunized animals or to develop ILTV as a polyvalent vaccine vector for avian respiratory tract disease. Our laboratory and others have identified an avian hepatoma cell line for the cultivation of ILTV (16,17). We have also elucidated the assembly pathway (18), determined the strength of four promoters for gene expression in avian hepatoma cells, documented the transactivation of the early SV40 promoter by ILTV co-infection (19), and constructed several recombinant ILTV with foreign genes inserted into genes coding for a serine-rich protein (7) or thymidine kinase (20). 5.5 kbp *Eco*RI

The nucleotide sequence data reported in this paper have been submitted to the GenBank nucleotide sequence database and have been assigned the accession number AF001078

fragment of the ILTV DNA was sequenced in searching for more insertion sites in constructing recombinant ILTV. A homology search of this DNA fragment identified a single potential open reading frame (ORF) encoding part of a subunit of a DNA helicase/primase complex.

Materials and Methods

Cells

A chicken hepatoma cell line was used for ILTV cultivation (16).

Isolation of ILTV DNA

The USDA challenge strain NSVL that was generously provided by Solvay Animal Health, Inc., was used in the DNA preparation. Adaptation of the virus to the hepatoma cell line was described previously (7).

ILTV DNA derived from cell culture supernatants of ILTV infected chicken hepatoma cells was used for *Eco*RI digestion and cloning as previously described (19).

*DNA Sequencing of the 5.5-kb ILTV-*Eco*RI Fragment*

To facilitate the sequencing, two libraries containing the subclones of the 5.5-kb DNA fragments were constructed. Library 1 was constructed by random shearing and library 2 by the use of two compatible restriction enzyme *Sau*3AI and *Bam*HI.

GENESIS 2000 automatic DNA analysis system (DuPont) and Sanger dideoxy chain termination method with the Sequenase-2.0 kit (USB) were used in DNA sequencing. DNA sequences obtained from the two libraries were assembled with the GeneWorks software (IntelliGenetics, Inc.) and further verified and extended in parallel by methods of GENESIS and Sanger using identical primers walking through the entire DNA fragment from both directions. If the same sequence was identified by both methods, the sequence was recorded. If the sequence was not identical, the analyses were repeated until there was agreement between the two methods.

Results and Discussion

Sequencing of the 5.5-kbp ILTV DNA Fragment

The complete nucleotide sequence of the 5,520-bp ILTV *Eco*RI DNA fragments has been deposited in GenBank with an accession number of AF001078.

Identification of a Gene Homologous to One Subunit of the DNA Helicase/Primase Complex

The DNA helicase/primase complex of HSV-1, which is essentail for DNA replication, consists of three sub-units with molecular weights of 12,000, 97,000 and 70,000, encoded by genes UL52, UL5 and UL8, respectively (21). The gene products of UL52 and UL5 are believed to be the two sub-units that are actually involved in DNA replication, and the UL8 gene has been reported to be required for efficient utilization of primers by polymerase, thereby

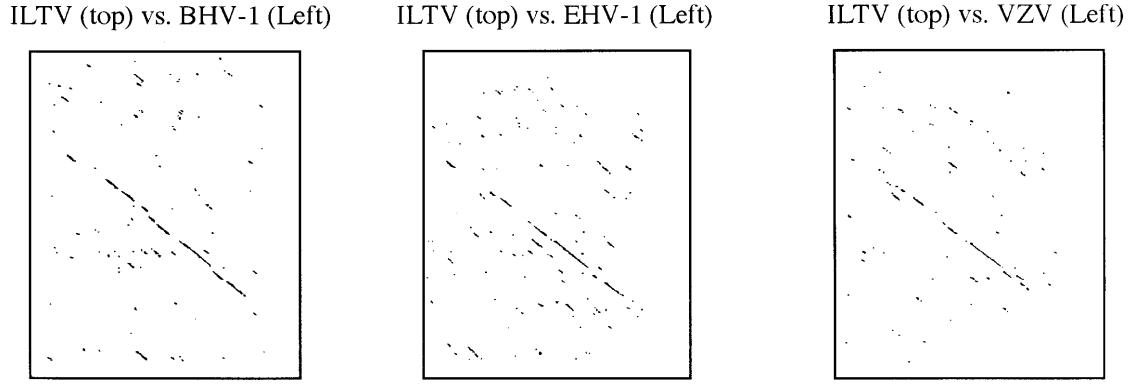

Fig. 1. Protein dot matrix showing similarity of the amino acid sequence of ORF of ILTV *Eco*RI DNA fragment to the UL8-equivalent genes of other herpesviruses with the GeneWorks analysis package (IntelliGenetics, Inc) (28). The top horizontal axis represents the ILTV DNA sequence, aligned with the DNA sequence from other alpha-herpesviruses (left vertical axis).

increasing the efficiency of lagging-strand synthesis (22). The UL52 and UL5-equivalent genes of ILTV have been reported (4,10), however, no information concerning the UL8 equivalent gene of ILTV is available. DNA analysis revealed one potential open reading frame (ORF) with 424 amino acids. This ORF was predicted to extend beyond this *Eco*RI fragment. Amino acid sequence comparison and homology search through GenBank with the Sequence Analysis Software Package of GCG (23) and BLAST (24) revealed that this ORF share sequence similarities to the UL8-equivalent gene of α-herpesviruses, that is, the ORF 52 of vericura-zoster virus (VZV), the ORF 54 of equine herpesvirus type-1 (EHV-1), a gene of bovine herpesvirus type-1 (BHV-1) that encoded protein associated with the DNA helicase/primase complex (Fig. 1), and to a lesser degree of similarity, our sequence also shared sequence identities to HSV-1 UL8 (21,25,26) and one subunit of DNA helicase/primase complex of canine herpesvirus (27) (data not shown).

Based on amino acid sequence identities it appears that thes genes are conserved between ILTV and other α-herpesviruses. Though the amino acid alignment provides considerable divergence in that they share low levels of conserved residues and high levels of conservative substitutions. The presence of these highly conserved genes in ILTV and other herpesviruses provides further evidence for the evolution of the α-herpesviruses from a common ancestor.

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