IDENTIFICATION AND STRUCTURAL ANALYSIS OF A MDV GENE ENCODING A PROTEIN KINASE

S.M. REDDY, D. SUI, P. WU, L. LEE

Avian Disease and Oncology Laboratory, USDA-ARS, 3606 East Mount Hope Road, East Lansing, MI 48823, USA

Summary. – DNA sequence analysis of the *Bam*HI-C fragment of Marek's Disease Virus (MDV) reveals the presence of a 513 amino acid open reading frame (ORF). This ORF codes for a protein with an estimated M_r of 58,901. Comparison of the amino acid sequence with those available in the Swiss-Prot database indicates extensive homology with a protein kinase (PK) of herpes simplex virus (HSV) and varicella-zoster virus (VZV). In Northern blot hybridization, a transcript of 2.0 kb was detected in MDV (GA strain) infected duck embryo fibroblasts (DEFs). A portion of the ORF was expressed in *Escherichia coli* as a *trp*E-fusion protein and used to generate antiserum in New Zealand rabbits. This antiserum specifically detected a protein of 60 kDa in MDV serotype 1, 2 and 3 infected DEFs or chicken embryo fibroblasts (CEFs) by Western blot analysis. This ORF codes for a functional PK.

Key words: MDV; protein kinase; UL13; nucleotide sequence

Introduction

MDV is a highly cell-associated avian herpesvirus. In chickens, MDV is the etiological agent of Marek's disease (MD), a malignant T-cell lymphoma (Calnek and Witter, 1991). MD has been largely controlled by vaccines that consisted of either live attenuated or naturally avirulent viruses (Witter, 1988). Knowledge of MDV genome structure and function is crucial to the understanding of viral oncogenicity and vaccine improvements. However, molecular analysis of MDV has lagged behind other herpesviruses because of the strong cell-associated nature of the virus. Some of the important glycoproteins of MDV with homology to HSV such as gB, gC, gD, gI, gH and gL have been identified (Ross *et al.*, 1989; Coussens and Velicer, 1988; Ross *et al.*, 1991; Scott *et al.*, 1993; Yoshida *et al.*, 1994). Of these glycoproteins, the gB, was shown to induce good protective immune response against MD (Nazerian *et al.*, 1992). Although recent progress in molecular biology of the virus has provided some knowledge on the structure and functions of the viral genome, very little is known on the enzyme systems involved in MDV replication and regulation.

PKs are involved in a wide variety of functions in eukaryotic cells including cellular metabolism, cell cycle control, hormonal responses and the control of transcription and translation (Hanks *et al.*, 1988). The study of structure and function of viral PK is essential for understanding of the viral DNA replication mechanism. PKs contain several highly conserved motifs within the consensus sequence of the 240 amino acid catalytic domain (Hanks *et al.*, 1988).

Recent sequence data on herpesviruses confirm the presence of a UL13 counterpart in the α-, β- and γ-herpesviruses and US3 counterpart only in the α-herpesviruses (McGeoch and Davison, 1986; Chee *et al.*, 1989; Smith and Smith, 1989; van Zijl *et al.*, 1990; Zhang *et al.*, 1990; deWind *et al.*, 1992; Colle *et al.*, 1992; Telford *et al.*, 1992; Nagesha *et al.*, 1993; Albrecht *et al.*, 1992). MDV US3 gene was recently identified as potentially encoding a PK by Ross *et al.* (1991), and was fount to be dispensable for virus growth in cell culture (Sakaguchi *et al.*, 1993).

In this study, we report the identification and sequence analysis of MDV homologue of HSV-1 UL13 gene. The MDV UL13 gene consists of a 1539 bp ORF capable of encoding a 513 aa protein with an estimated M_r of 58,901. Transcriptional analysis indicated that the MDV UL13 message is 2.0 kb. In addition, PK assay indicated that the MDV UL13 gene product has functional PK activity.

Materials and Methods

Cells and viruses. GA strain of MDV-1 was propagated in DEFs and MDV strains Rispens (serotype 1), SB-1 (serotype 2) and HVT (serotype 3) were propagated in CEFs. Both DEFs and CEFs were grown in Leibovitz-McCoy's medium (GIBCO Laboratories) supplemented with 4% calf serum at 37°C (Eidson *et al.*, 1968; Solomon, 1975).

DNA sequencing and computer analysis. A 4.8 kbp *Kpn*I fragment from the *Bam*HI-C fragment of MDV-GA DNA in pACYC184 vector (Fukuchi *et al.*, 1984) was cloned into the *Kpn*I site of pUC18. The sequencing strategy employed a combination of sequential deletions, using exonuclease III and mung bean nuclease (Henikoff, 1984). Both strands of the *Kpn*I fragment were sequenced by the dideoxynucleotide chain termination method using [^{35}S]dATP (NEN) and the TAQuence version 2.0 DNA sequencing kit (United States Biochemical Corp.) as suggested by the manufacturer. The complete sequence was determined by analysis of overlapping clones. Potential ORFs, homology with other sequences available in databases, and pairwise percentage identities and similarities were examined using the MAP, BLAST and GAP programs, respectively, of the University of Wisconsin Genetics Computer Group package (Devereux *et al.*, 1984). Sequence alignment of UL13 of MDV and other two herpesviruses was done by the clustal method using the Megalign program of the DNAstar package.

Northern blot hybridization. Total cellular RNA from mock- and MDV GA-infected DEFs was isolated at 48 hrs post infection as described previously (Sambrook *et al.*, 1989). Total RNA (20 µg) was loaded onto 1.2% agarose gels containing 6% formaldehyde and electrophoretically separated and transferred onto a Hybond-N membrane. Northern blot hybridization was performed using standard procedures (Sambrook *et al.*, 1989). A 951 bp DNA probe from a *Hind*III-*Sph*I fragment of the 4.8 kbp *Kpn*I fragment was radiolabeled with [α-^{32}P]dCTP using a random priming method (Prime-It, Stratagene). Blots were denatured in 0.05 mol/l NaOH for 15 mins and neutralized in 6 x SSC solution for 45 mins. After baking the blots for 2 hrs at 80°C, they were prehybridized in standard prehybridization solution containing 50% formamide at 42°C. The blots were than hybridized with the probe overnight at 42°C and analyzed by autoradiography.

Cloning, expression, and preparation of antiserum against trpE-PK fusion protein. The pATH vector, which encodes the *trp*E gene transcribed by the inducible *trp* promoter (Koerner *et al.*, 1991), was used to express the MDV UL13 gene in *E. coli*. The entire ORF of MDV UL13 was PCR-amplified. To generate pATH MDV UL13 PK, the entire ORF (1,543 bp) of MDV UL13 was cloned into pATH11. The *trp*E-UL13 fusion protein was produced as described previously (Koerner *et al.*, 1991) and was partially purified by incubating the expressing bacteria at 65°C in the presence of 1% SDS. Bacterial lysates were centrifuged for 15 mins at 3000 x g, and the resulting pellet containing the fusion protein was dissolved in 2% SDS and used for immunizations. Two hundred µg of the *trp*E-UL13 fusion protein was emulsified in complete Friend's adjuvant (GIBCO) and injected into one New Zealand white rabbit. Four and 8 weeks post inoculation, the rabbit was given booster injections [200 µg of the antigen emulsified in incomplete Freund's adjuvant (GIBCO)]. The rabbit was bled 10 days after the second booster injection, and the serum was used for immunoprecipitation and Western blot analysis.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Mock- and MDV-infected DEF and CEF cell lysates were prepared with triple-detergent lysis buffer (Sambrook *et al.*, 1989). Lysates were separated by SDS-PAGE (10% gel) and transferred to a nitrocellulose membrane (NC) (Sleicher & Schuel). The membranes were blocked using 5% dry non-fat milk. Rabbit antiserum against the *trp*E-UL13 fusion protein was used at a 1:100 dilution and donkey anti-rabbit IgG conjugated with horseradish peroxidase was used as a secondary antibody. The positive polypeptides on membranes were detected with chemiluminescence substrate (Amersham Lifescience, UK).

PK assay. Mock- and MDV-infected cell lysates were pre-cleared with 15 µl of rabbit pre-immune serum for 45 min and then with protein-A Sepharose beads for another 45 mins. The precleared lysates were then immunoprecipitated with the rabbit antiserum against the *trp*E-UL13 fusion protein. The immune complexes were captured using protein A-conjugated-Sepharose beads and washed three times with lysis buffer and twice with kinase buffer (50 µmol/l Tris-HCl pH 8.0, 50 mmol/l $MgCl_2$, 0.1% NP40 and 1 mmol/l DTT). The PK activity assay was performed by incubating the immune complexes with 50 µl of PK buffer supplemented with 15 µCi of [γ-^{32}P] ATP at 37°C for 40 mins. The beads were washed three times in lysis buffer and spotted onto Whatman 3M filter discs. The filter discs were washed twice for 10 min in 10% (w/v) trichloroacetic acid (TCA), three times in 5% TCA, and twice in absolute ethanol, and then dried. Incorporation of radioactivity was measured in a liquid scintillation counter.

Results

DNA sequence analysis of MDV PK gene

A 4.8 kbp *Kpn*I fragment from MDV-GA *Bam*HI-C fragment (15 kbp) was sequenced. An overall map of the corresponding genomic region is shown in Fig. 1. Computer-aided analysis identified three ORFs homologous to HSV-1. These ORFs were homologous to HSV-1 UL12, UL13, and UL14. The MDV UL13 gene was found to be 1869 bp in length. The sequences upstream and downstream of the (UL13) PK gene were analyzed for the putative transcriptional control elements. A consensus „TATA" box, characteristic of many eukaryotic and herpesviral promoters (Breathnach and Chambon, 1981; Cordon *et al.*, 1980; Wagner, 1985), was found –69 to –65 nt from the proposed initiation codon (data not shown). A promoter motif (CAATTAG) related to transcription control was located upstream from the ATG codon at position of –134 to –129, and exhibits similarity to the „CAT" box consensus sequence. Two polyadenylation signal sequences, „AAATATT" and „AAATTATA" lie downstream from the ORF, at position of 1934 to 1940 and 1944 to 1951 (data not shown) (Breathnach and Chambon, 1981; Cordon *et al.*, 1980; Wagner, 1985). The

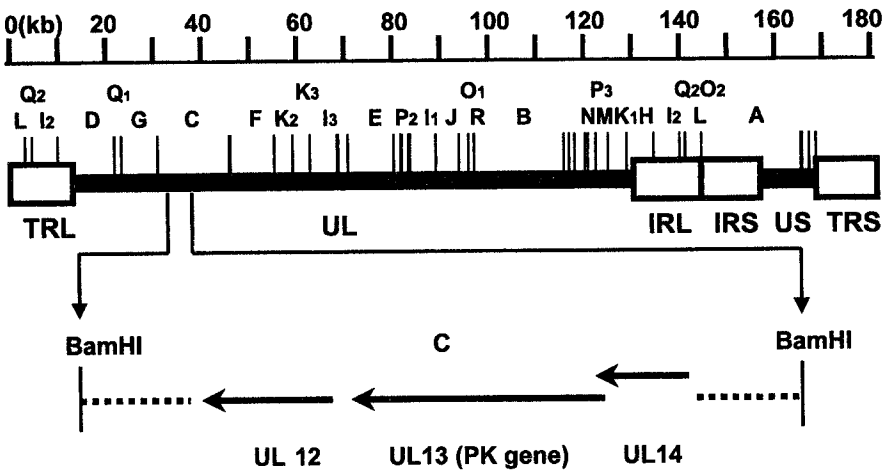

Fig. 1
Location of the UL13 (PK) homologue gene in the MDV genome
The upper part of the figure gives schematic representation of the MDV genome. TRL, long terminal repeat; UL, unique long region; IRL, long terminal repeat; IRS, long internal repeat; US, unique short region. The lower part defines the *BamHI* restriction map (Fukuchi *et al.*, 1985). Arrows delineate the locations of the three ORFs and their transcriptional directions.

1539-bp ORF is capable of coding for a 513 aa polypeptide with an estimated of M_r of 58,901.

Sequence analysis of MDV UL13 PK

In order to study the structural relationship of α-herpesvirus PKs, the predicted coding regions of PKs of MDV, HSV-1 (Knopf, 1986) and VZV (Davison, 1986) were compared by the clustal method. The size of MDV PK was 513 aa compared to 518 aa in HSV-1 and 510 aa in VZV. There was 31% identity and 41% similarity between UL13 homologues of MDV, HSV-1 and VZV (Table 1). Phylogenetic analysis of PKs of α-, β- and γ-herpesviruses using Clustal method is shown in Fig. 3.

The PK catalytic domains contain several motifs, which form a distinguishing pattern (Hanks *et al.*, 1988). These domains are also conserved in MDV PK (Fig. 2). The consensus motif number I is highly conserved and in addition to PKs is also found in nucleotide binding proteins (Wierenga and Hol, 1983). Regions II and VIII, which correspond to the catalytic domains, are also conserved in MDV UL13 PK. Similarly, all other motifs are highly conserved when compared to other known protein kinases, suggesting that MDV UL13 gene codes for a serine/threonine PK.

Transcription analysis of MDV PK gene in infected cells

Northern blot hybridization was performed in order to detect the transcript of MDV UL13 PK in MDV-infected cells. The estimated size of the MDV-UL13 PK transcript (from TATA promoter to the poly-A signal) was 1869 nt. As shown in Fig. 4, lane 2, a single transcript about 2.0 kb was detected. This transcript, therefore, corresponds to the MDV UL13 PK gene mRNA.

Table 1. Identities/similarities among UL13 PK homologues of α-herpesviruses

	MDV	EHV	VZV
EHV	37/46		
VZV	31/41	38/46	
HSV	31/42	33/42	32/41

Per cent values.

Identification of the PK in infected cells

The three serotypes of MDV were analyzed for expression of UL13 PK. Western blot analysis of cells infected with all three MDV serotypes using rabbit anti-trpE-UL13 fusion protein polyclonal serum specifically detected a 60 kDa protein. The M_r of the detected protein is in agreement with the calculated value. In order to determine if MDV UL13 has PK activity, cold lysates of mock- and MDV-infected DEF cells were subjected to PK assay. PK activity of the different samples was measured by monitoring incorporation of [γ-^{32}P]ATP into TCA-precipitable material. Immunoprecipitation of mock-infected cells gave 2,400 cpm compared to 26,300 cpm for GA-infected cells indicating that MDV UL13 gene product presents PK activity.

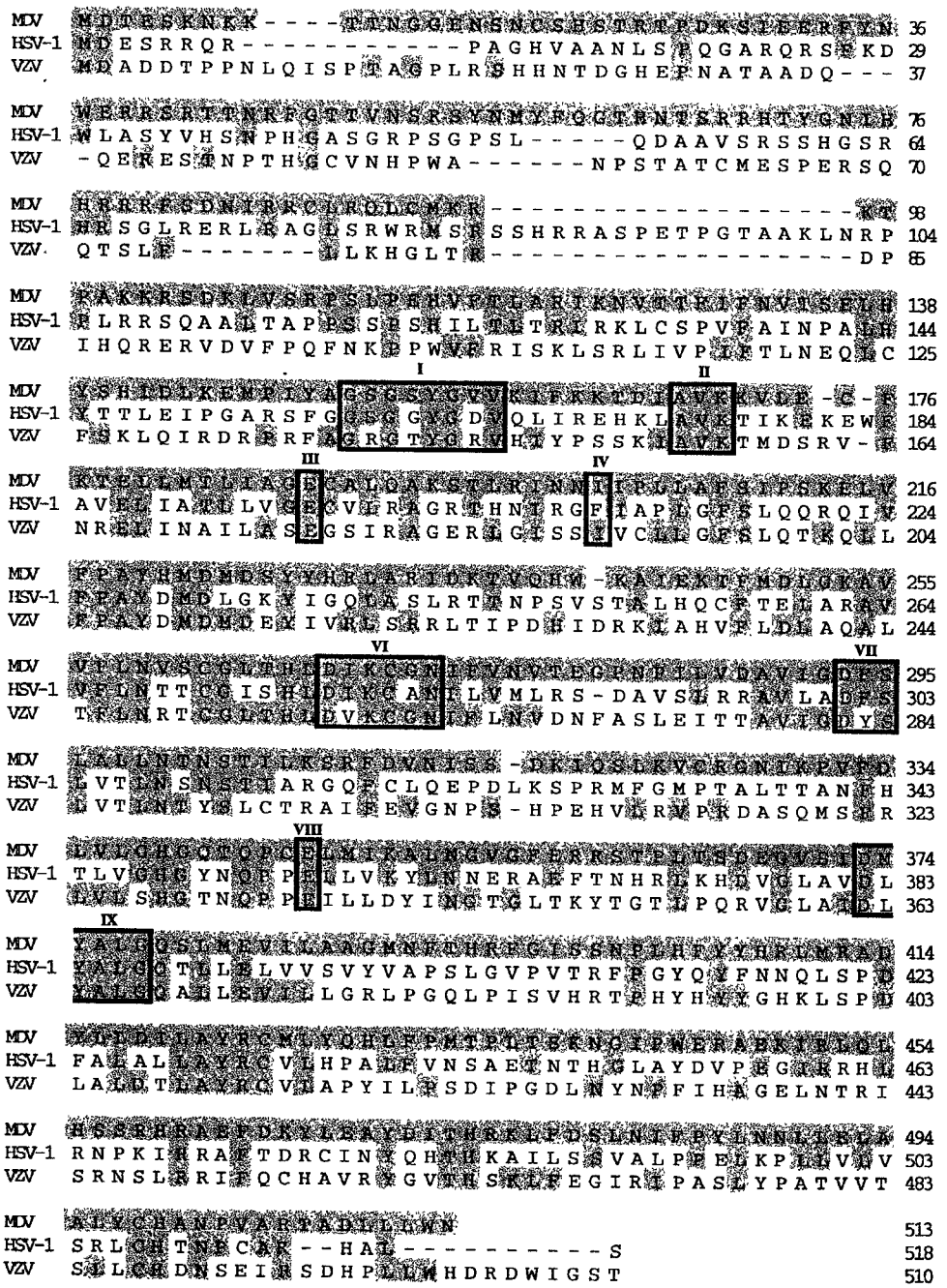

Fig. 2
Comparison of amino acid sequences of PKs α-herpesviruses
UL13 homologues of MDV, HSV-1 and VZV were compared using Megalign program of DNAs tar (identical aa are shaded). Conserved motifs are boxed.

Discussion

HSV-1 codes for three PKs: US3, UL13, and ribonucleotide reductase (Purves *et al.*, 198; Paradis *et al.*, 1991; Smith and Smith, 1989). The two major HSV PKs, UL13 and US3, have been shown to be non-essential for virus replication *in vitro* (Coulter *et al.*, 1993; Purves *et al.*, 1987). In MDV, the US3 homologue has also been report-

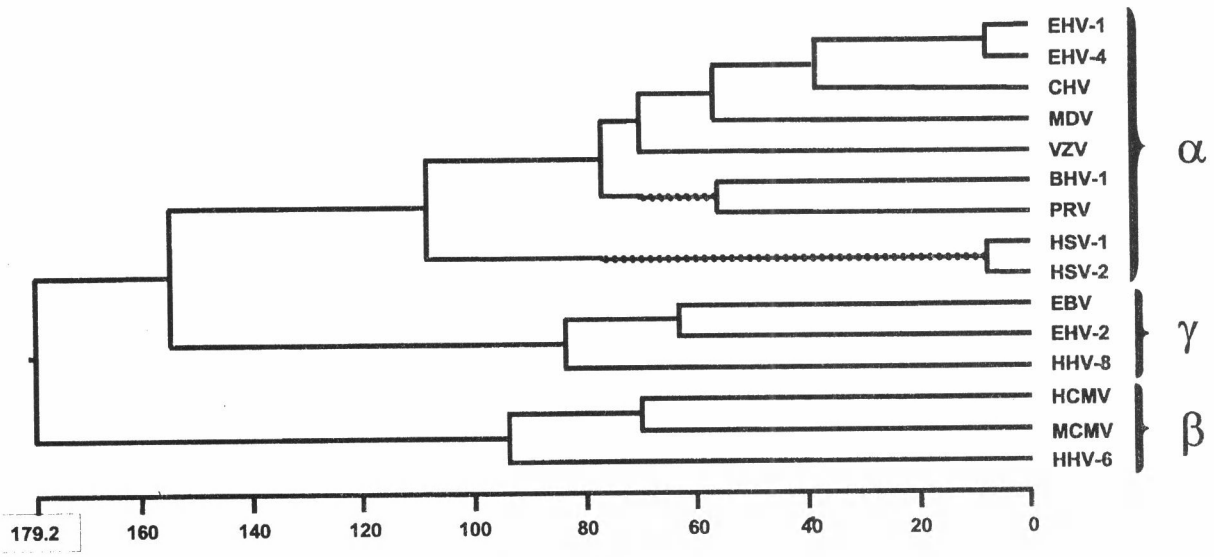

Fig. 3
Phylogenetic tree based on the PK sequence alignments
The tree was constructed from alignments of 15 herpesvirus PK sequences using the clustal method of Megalign program (DNAstar).

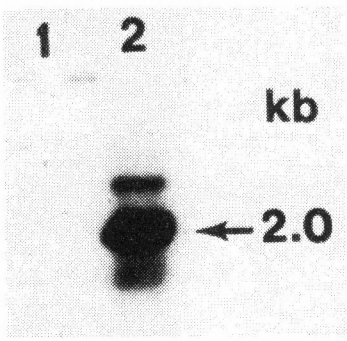

Fig. 4
Northern blot analysis
Total RNA from MDV- (GA strain) and mock-infected DEF cells was isolated at 48 hrs post infection, electrophoresed (20 μg) and blotted. The blot was hybridized with a MDV UL13-specific probe. The size of the mRNA was calculated according to the RNA size standard. Lane 1: mock-infected DEF cells; lane 2: MDV-infected DEF cells.

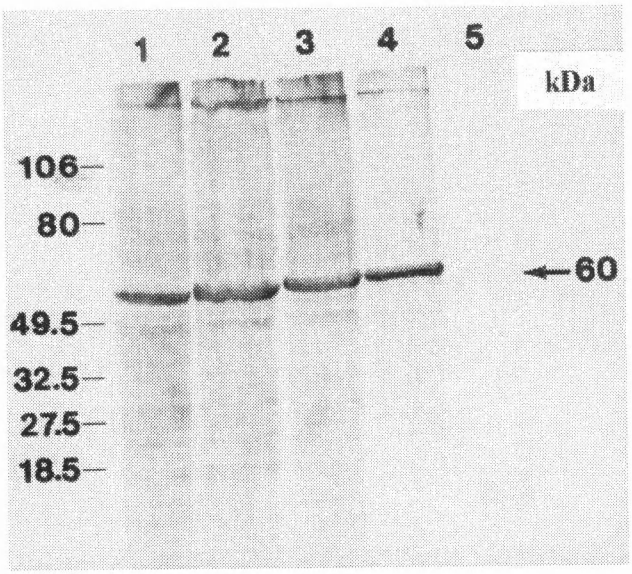

Fig. 5
Western blot analysis
DEF cells were infected with MDV-GA and CEF cells with MDV-Rispen, HVT, and MDV-SB-1, harvested 48 hrs post infection and lysed. The lysates were separated by SDS-PAGE (10% gel) and subjected to Western blot analysis using rabbit anti-trpE-MDV UL13 fusion protein serum. MDV-GA (lane 1), MDV-Rispen (lane 2), HVT (lane 3), MDV-SB-1 (lane 4), and uninfected CEFs (lane 5). The arrow indicates the UL13-specific band.

ed and was shown to be non-essential for virus replication (Sakaguchi *et al.*, 1993), however, UL13 homologue has not yet been identified. In the present study, we have identified and characterized the gene for a PK of the GA strain of MDV. The gene is located in the *Bam*HI-C fragment of the MDV genome UL region, and has significant homology to HSV-1 UL13 PK. Sequence analysis of the MDV UL13 PK indicates that the gene contains 1539 bp encoding a polypeptide of 513 aa. Transcriptional analysis of HSV-1 indicated that the UL13 transcript is coded by a family of overlapping transcripts (Costa *et al.*, 1983) and

was found to be 3.9 kb in size. However, the MDV UL13 mRNA was shown to be 2.0 kb in size. This size of mRNA is potentially capable of encoding the entire PK polypeptide assuming no splicing occurs.

UL13 protein of MDV consists of 513 aa compared to 518 aa in HSV-1 and of 510 aa in VZV. The amino acid homology between MDV and EHV-1 PKs was 37%, but was only 31% when compared to VZV or HSV-1. This data indicate that EHV-1 PK is more closely related to MDV PK than VZV or HSV-1 PKs. The ORFs of several PK genes have been sequenced and analyzed for their amino acid sequence patterns. Hanks *et al.* (1988) have reported several conserved domains in the catalytic domains of PKs. Conserved motif VI suggests that MDV UL13 PK is probably a serine/threonine PK, whereas motifs I and VII are thought to be involved in ATP binding. Motifs II and VIII are involved in catalytic reaction. MDV PK also shows extensive amino acid homology with the other herpesviruses PKs. Phylogenetic analysis of the deduced amino acid sequences from herpesvirus PKs suggests that evolutionarily, α-herpesvirus are more closely related to γ- than β-herpesviruses.

Western blot analysis showed that the UL13 PK has an apparent size of 60 kDa in agreement with the calculated M_r of 58,901. It was shown that PK is expressed in infected cells by all three MDV serotypes.

Like its α-herpesvirus homologues, MDV UL13 possesses PK activity. In the presence of [γ^{32}P] ATP, we observed that MDV UL13 is able to phosphorylate polypeptides. This data suggests that MDV UL13 codes for a functional PK. In HSV, it is known that UL13 is responsible for phosphorylation of viral and cellular proteins (Ng *et al.*, 1998; Kawaguchi *et al.*, 1998). It would be interesting to study the role of MDV UL13 in virus replication and phosphorylation of viral Fc receptor.

Acknowledgements. We thank Larry Bacon for reviewing the manuscript, and Barry Coulson for technical assistance.

References

Albrecht JC, Nichlas J, Biller D, Cameron KR, Biesinger B, Newman C, Wittmann S, Craxton MA, Coleman H, Fleckenstein B, Honess RW (1992): Primary structure of the herpesvirus saimiri genome. *J. Virol.* **66**, 5047–5058.

Baer R, Bankier AT, Biggin MD, Deininger PL, Farrell PJ, Gibson TJ, Hatfull G, Hudson GS, Satchwell SC, Seguin C, Tuffnell PS, Barrel BG (1984): DNA sequence and expression of B95-8 Epstein-Barr virus genome. *Nature* **310**, 207–211.

Breathnach R, Chambon P (1981): Organization and expression of eukaryotic split genes coding for proteins. *Annu. Rev. Biochem.* **50**, 349–383.

Buckmaster AE, Scott SD, Sanderson MJ, Boursnell ME, Ross NL, Binns MM (1988): Gene sequence and mapping data from Marek's disease virus and herpesvirus of turkeys: implications for herpesvirus classification. *J. Gen. Virol.* **69**, 2033–2042.

Calnek BW, Witter RL (1991): Marek's disease. In Calnek BW, Barnes HJ, Beard CW, Reid WM, Yoder HW Jr (Eds): *Diseases of Poultry*. Iowa State University Press, Ames, pp. 342–385.

Chee MS, Lawrence GL, Barrell BG (1989): Alpha-, beta- and gammaherpesviruses encode a putative phosphotransferase. *J. Gen. Virol.* **70**, 1151–1160.

Chen WS, Lazar CS, Poenie M, Tsien RY, Gill GN, Rosenfeld MG (1987): Requirement for intrinsic protein tyrosine kinase in the immediate and late actions of the EGF receptor. *Nature* **328**, 820–823.

Colle CF, Flowers CC, O'Callaghan DJ (1992): Open reading frames encoding a protein kinase, homolog of glycoprotein gX of pseudorabies virus, and a novel glycoprotein map within the unique short segment of equine herpesvirus type 1. *Virology* **188**, 545–575.

Cordon J, Wasylyk B, Buckwalder A, Sassome-Corsi P, Kedinger C, Chambon P (1980): Promoter sequence of eucaryotic protein coding genes. *Science* **209**, 1406–1414.

Costa RH, Draper KG, Banks L, Powell KL, Cohen G, Eisenberg R, Wagner EK (1983): High-resolution characterisation of herpes simplex virus type 1 transcript encoding alkaline exonuclease and a 50,000-dalton protein tentatively identified as a capsid protein. *J. Virol.* **48**, 591–603.

Coulter LJ, Moss HWM, Lang J, McGeoch DJ (1993): A mutant of herpes simplex virus type 1 in which the UL13 protein kinase gene is disrupted. *J. Gen. Virol.* **74**, 387–395.

Coussens PM, Velicer LF (1988): Structure and complete nucleotide sequence of the Marek's disease herpesvirus *gp* 57–65. *J. Virol.* **62**, 2373–2379.

Davison AJ, Scott JE (1986): The complete DNA sequence of Varicella-Zoster virus. *J. Gen. Virol.* **67**, 1759–1816.

Devereux J, Haeberli P, Smithies O (1984): A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res.* **12**, 387–395.

DeWind N, Domen J, Brens A (1992): Herpesviruses encode an unusual protein-serine/threonine kinase which is nonessential for growth in cultured cells. *J. Virol.* **66**, 5200–5209.

Eidson CS, Schmittle SC (1968): Studies on acute Marek's disease. I. Characteristics of isolate GA in chickens. *Avian Dis.* **12**, 467–476.

Fukuchi K, Sudo M, Lee YS, Tank A, Nonoyama M (1984): Structure of Marek's disease virus DNA: detailed restriction enzyme map. *J. Virol.* **51**, 102–109.

Hanks SK, Quinn AM, Hunter T (1988): The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. *Science* **241**, 42–52.

Henikoff S (1984): Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. *Gene* **28**, 351–359.

Kawaguchi Y, van Sant C, Roizman B (1998): Eukaryotic elongation factor 1delta is hyperphosphorylated by the protein

kinase encoded by the UL13 gene of herpes simplex virus 1. *J Virol.* **72,** 1731–1736.

Koerner TJ, Hill JE, Myers AE, Tzagoloff A (1991): High-expression vectors with multiple cloning sites for construction of trp E fusion genes: pATH vectors. *Methods Enzymol.* **194**, 477–490.

Kogan PH, Blissard GW (1994): A baculovirus gp 64 early promoter is activated by host transcription factor binding to CACGTG and GATT elements. *J. Virol.* **68,** 813–822.

Laemmli UK (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685.

McGeoch DJ, Dalrymple MA, Davison AJ, Dolan A, Frame MC, Mcnab D, Perry LJ, Scott JL, Taylor P (1988): The complete DNA sequence of the long unique region in the genome of herpes simplex virus type 1. *J. Gen. Virol.* **96**, 1531–1574.

McGeoch DJ, Davison AJ (1986): Alphaherpesviruses possess a gene homologous to the protein kinase gene family of eukaryotes and retroviruses. *Nucleic Acids Res.* **14**, 1765–1777.

Nagesha HS, Crabb BS, Studdert MJ (1993): Analysis of the nucleotide sequence of five genes at the left end of the unique short region of the equine herpesvirus 4 genome. *Arch. Virol.* **128**, 143–154.

Nazerian K, Lee LF, Yanagida N, Ogawa R (1992): Protection against Marek's disease by a fowlpox virus recombinant expressing the glycoprotein B of Marek's disease virus. *J. Virol.* **66**, 1409–1413.

Ng TI, Ogle WO, Roizman B (1998): UL13 protein kinase of herpes simplex virus 1 complexes with glycoprotein E and mediates the phosphorylation of the viral Fc receptor: glycoproteins E and I. *Virology* **241,** 37–48

Paradis H, Gaudreau P, Massie B, Lamarche N, Guilbault C, Gravel S, Langelier Y (1991): Affinity purification of active subunit 1 of herpes simplex virus type 1 ribonucleotide reductase exhibiting a protein kinase activity. *J. Biol. Chem.* **266**, 9647–9651.

Payne LN (1989): Pathogenesis of Marek's disease virus. In Kato S, Horiuchi T, Mikami T, Hirai K (Eds): *Advances in Marek's Disease Virus Research.* Japanese Association on Marek's Disease, Osaka, pp. 307–316

Purves FC, Longnecker RM, Leader DP, Roizman B (1987): Herpes simplex virus 1 protein kinase is encoded by open reading frame US3 which is not essential for virus growth in cell culture. *J. Virol.* **61**, 2896–2901.

Ross LJN, Sanderson M, Scott SD, Binns MM, Doel T, Milne B (1989): Nucleotide sequence and characterization of the Marek's disease virus homologue of glycoprotein B of herpes simplex virus. *J. Gen. Virol.* **70**, 1789–1804.

Ross LJN, Binns MM, Pastorek J (1991): DNA sequence and organization of genes in a 5.5 kbp EcoRI fragment mapping in the short unique segment of Marek's disease virus (strain RB1B). *J. Gen. Virol.* **72**, 949–954.

Sakaguchi M, Urakawa T, Hirayama Y, Miki N, Yamamoto M, Zhu GS, Hirai K (1993): Marek's disease virus protein kinase gene identified within the short unique region of the viral genome is not essential for viral replication in cell culture and vaccine-induced immunity in chickens. *Virology* **195**, 140–148.

Sambrook J, Fritsch EF, Maniatis T (1989): *Molecular Cloning: A Laboratory Manual.* 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor.

Scott SD, Smith GD, Ross LJN, Binns MM (1993): Identification of sequence analysis of the homologues of the herpes simplex virus type 1 glycoprotein H in Marek's disease virus and the herpesvirus of turkeys. *J. Gen. Virol.* **74**, 1185–1190.

Silva RB, Lee LF (1984): Monoclonal antibody-mediated immuno-precipitation of protein from cells infected with Marek's disease virus or turkey herpesvirus. *Virology* **136**, 307–320.

Smith RF, Smith TF (1989):Identification of new protein kinase-related genes in three herpesviruses, herpes simplex virus, varicella-zoster virus, and Epstein-Barr virus. *J. Virol.* **63**, 450–455.

Solomon JJ (1975): Preparation of avian cell cultures. *Tissue Culture Association* **1**, 7–11.

Telford EA, Watson MS, Mcbride K, Davison AJ (1992): The DNA sequence of equine herpesvirus 1. *Virology.* **189**, 304–316.

van Zijl M, Van der Gulden H, de Wind N, Gielkens A, Berns A (1990): Identification of two gene in the unique short region of pseudorabies virus: comparison with herpes simplex virus and varicella-zoster virus. *J. Gen. Virol.* **71**, 1747–1755.

Wagner EK (1985): Individual HSV transcription characterization of specific genes. In Roizman B (Ed.): *The Herpesviruses.* Vol 3. Plenum Press, New York, pp. 45–104.

Wierenga RK, Hol WG (1983): Predicted nucleotide-binding properties of p21 protein and its cancer-associated variant. *Nature* **302,** 842–844

Witter RL (1988): Advances in Marek's Disease Research. In Kato S, Horiuchi T, Mikami Y, Hirai K (Eds.): *Proc. 3rd Int. Sym. Marek's Dis.,* Osaka, pp. 389–397.

Yoshida S, Lee LF, Yanagida N, Nazerian K (1994) Identification and characterization of a Marek's disease virus gene homologous to glycoprotein L (gL) of herpes simplex virus type 1. *Virology* **204**, 414–419.

Zhang G, Stevens R, Leader DP (1990): The protein kinase encoded in the short unique region of pseudorabies virus: description of the gene and identification of its product in virions and in infected cells. *J. Gen. Virol.* **71**, 1757–1765.