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Structural organization and analysis of the viral terminase gene locus of Tupaia herpesvirus☆

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Abstract

Tupaia herpesvirus (THV) was isolated from spontaneously degenerating tissue cultures of malignant lymphoma, lung, and spleen cell cultures of tree shrews (*Tupaia* spp.). In order to determine the phylogenetic relatedness of THV the complete nucleotide sequence of the viral terminase (VTER) gene locus (6223 bp) of Tupaia herpesvirus strain 2 (THV-2) was elucidated and analysed. The VTER gene locus, encoding one of the most highly conserved herpesviral proteins is composed of two exons. The intron contains five potential open reading frames (ORFs). The arrangement of these ORFs is collinear with the corresponding regions in the genomes of the mammalian cytomegaloviruses. The precise primary structure of the THV-2 VTER splice junction was determined using RT-PCR and was found to be in agreement with the corresponding splice donor and acceptor sites of the mammalian cytomegaloviruses. The comparison of all six putative THV-2 proteins with the corresponding counterparts in other herpesviruses revealed that THV resides between the Human and the Murine cytomegalovirus (HCMV, MCMV). These results are in agreement with our previous statement, that THV and the known cytomegaloviruses are closely related to each other and should be classified into one taxonomic group. The genetic data presented here and in previous studies are based on the detailed comparison of highly conserved viral genes. Consequently, the classification of the Human and the cytomegaloviruses into the two genera *Cyto-* and *Muromegalovirus*, that is mainly based on overall genome structure, should be reconsidered.

Keywords: Tupaia herpesvirus; Herpesviridae; RT-PCR; Nucleotide sequence analysis; Terminase; Tree shrew

1. Introduction

Tupaia herpesvirus (THV) infects tree shrews (*Tupaia* spp., family Tupaiidae), a group of primitive higher mammals (Proteutheria, Scandentia), which is thought to have diverged at the base of the primate evolutionary tree (Novacek, 1992; Kumar and Hedges, 1998). After the first discovery of herpesvirus-like particles (THV strain 1) in a spontaneously degenerating lung tissue culture of a tree shrew (Mirkovic et al., 1970; McCombs et al., 1971), six additional THV isolates were subsequently established. These viruses termed

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THV-1 to 7 were isolated from malignant lymphoma tissue cultures and degenerating spleen cell cultures of tree shrews (Darai et al., 1979, 1981, 1983; Darai and Koch, 1984; Koch et al., 1985). THV particles show the classical morphology of herpesviruses (McGeoch, 1989; Roizman, 1996) and contain a linear double-stranded DNA genome of about 200 kbp in size (Darai et al., 1981; Koch et al., 1985). The seven isolates of THV were grouped into five THV strains according to their restriction endonuclease cleavage patterns. Molecular cloning and physical mapping of the genome of Tupaia herpesvirus strain 2 (THV-2) was performed and a complete genome library was established (Koch et al., 1985).

THV, as well as the murine, rat, guinea pig, and simian cytomegaloviruses, belongs to genome group F (Roizman et al., 1981) and is characterized by a unique DNA sequence without any extended repeats resulting in the absence of different genomic isomers.

Recently THV was classified into the subfamily Betaherpesvirinae by characterization of a highly conserved gene cluster of herpesviruses coding for the DNA polymerase (DPOL), glycoprotein B (gB), a probable processing and transport protein (PRTP), and the major DNA binding protein (DNBI). Phylogenetic analysis of these four genes revealed a perfect colinearity and a high relatedness of THV with the Murine and the Human cytomegalovirus (MCMV, HCMV) (Springfeld et al., 1998; Bahr et al., 1999). This finding makes it necessary to elucidate other highly conserved herpesviral genes. The herpesviral terminase (VTER) gene locus which comprises two exons and a semiconserved intron sequence was found to be a suitable target gene because it is exceptionally highly conserved among the familiy Herpesviridae. Recently, Bogner et al. (1998) demonstrated that HCMV VTER binds HCMV PRTP raising the possibility that these two proteins form the heterodimeric viral terminase protein complex, which is in analogy to a well-studied process in bacteriophages [e.g. gp16/gp17 of Bacteriophage T4 (Kuebler and Rao, 1998; Lin et al., 1997)]. VTER is an essential factor that is required to cleave large concatemeric replication intermediates into genome-sized monomers (Deiss et al., 1986). The identification and characterization of the VTER gene locus of THV is the subject of the present report.

2. Methods

2.1. Viral DNA and genomic library

The propagation of THV was carried out as described previously (Darai et al., 1983). The recombinant plasmids harboring specific DNA sequences of the THV-2 genome were obtained from a defined genome library as described elsewhere (Koch et al., 1985).

2.2. Enzymes and DNA isolation

The restriction endonucleases and T4 DNA ligase were purchased from Roche Diagnostics GmbH (Mannheim, Germany). Incubations were carried out according to standard procedures for each enzyme. DNA fragments were isolated from 0.8% low melting point agarose gels as described previously (Koch et al., 1985). The recombinant plasmids harboring the DNA sequences of the *Eco*RI fragments B, L, and F of THV-2 were purified using the Qiagen Plasmid Midi Prep procedure (Qiagen GmbH, Hilden, Germany). The PCR products were purified using Micro-Spin S-300 HR columns (Pharmacia Biotech, Germany).

2.3. DNA sequencing

The recombinant plasmids and the purified PCR products were sequenced using the DyeDeoxy Terminator *Taq* cycle sequencing technique (Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit, Applied Biosystems, Weiterstadt, Germany) and a 373A "Extended" DNA sequencer (Applied Biosystems, Weiterstadt, Germany) as described previously (Tidona and Darai, 1997). The nucleotide sequence of the VTER gene locus was determined by primer walking. The Sequence Navigator software (Version 1.01, Applied Biosystems GmbH, Weiterstadt, Germany) was used to assemble the nucleotide sequences obtained from individual sequencing reactions.

2.4. Reverse transcriptase polymerase chain reaction

Tupaia baby fibroblasts (TBF) were infected with THV-2 in the presence or absence of inhibitory agents. Total cellular RNA was isolated at 4, 7, 10, and 14 h post infection to obtain immediate-early (IE, in the presence of cycloheximide), early (E, in the presence of cytosine arabinoside), early-late (EL), and late (L) viral transcripts, respectively. RNA isolation was performed using the guanidinium/cesium chloride method as described previously (Rösen-Wolff et al., 1988). The reverse transcription step was carried out using the RNA LA PCR Kit Ver. 1.1 (Takara Shuzo Co, Shiga, Japan). PCR was performed using 0.5 fmol of the template DNA in 100 μl volumes containing 1.5 mM $MgCl_2$, 12.5 nmol of each dNTP, 50 pmol of each primer, and 2.5 units of *ExTaq* DPOL (Takara Shuzo Co, Shiga, Japan). A total of 35 cycles were run in an automated temperature cycling reactor (Genius, Techne, Cambridge, UK) under cycling conditions of 96°C for 30 s, 60°C for 1 min, and 72°C for 2 min per cycle. Specific oligonucleotide primers were designed in order to amplify the coding region around the splice site of the viral terminase gene of THV-2. The following PCR primers were used in the RT-PCR experiment: 5′-AAGCACGTTTCCCAGTTCGTCC-3′ (Primer 1) and 5′-GAGTTTGGTCAGGAAGCAGGTG-3′ (Primer 2). The primer 2 was used for the first strand synthesis of the cDNA by R reaction.

2.5. Computer-assisted analysis

Nucleotide and amino acid sequences were compiled and analysed by using the PC/GENE program release 6.85 (Intelligenetics Inc, Mountain View, CA, USA). Protein alignments were carried out with the CLUSTAL program (Higgins and Sharp, 1988). The phylogenetic tree was calculated using the PC programs ClustalW (Ver.1.64b; Thompson et al., 1994), Kitsch (Ver. 10.0), Protdist (Ver. 10.0), and Treeview (Ver. 1.5.2).

3. Results

3.1. Analysis of the nucleotide sequence between map coordinates 0.730 and 0.763 of the THV genome

In this study the 6223 bp long nucleotide sequence between the genome coordinates 0.730 and 0.763 of THV-2 was determined by automated cycle sequencing and primer walking. This THV-2 genome region includes the complete *Eco*RI-fragment L (5425 bp) and short terminal sections of the *Eco*RI-fragments B (708 bp) and F (93 bp) (Fig. 1A). Both DNA strands were sequenced independently and each nucleotide was determined with an average redundancy of 2.57. The G + C content of this particular genome region was presumed to be 67.54%. Seven potential open reading frames (ORFs) of 1125, 648, 342, 687, 1623, 1083, and 894 nucleotides (nt), were identified within the 6223 bp long DNA sequence (Fig. 1A). Computer-assisted analysis of the deduced amino acid sequences of these seven ORFs revealed that the two flanking ORFs of this particular genome region of THV-2 showed the highest homologies with the two exons of the conserved terminases (VTER) of other herpesviruses. Four (T91–T94) out of the five ORFs within the intron of the VTER gene showed the highest homologies to the gene products of UL91–UL94 of the HCMV strain AD169 and M91–M94 of the MCMV strain Smith, whereas the fifth ORF (T90) revealed no homology at all with any known herpesviral protein. The seven THV-2 ORFs, located at this particular genome region, form a dense and partially overlapping gene cluster. The overall arrangement, transcription directions and sizes of these ORFs are perfectly collinear with HCMV and MCMV, with exception of ORFs T90, UL90, and m90, that are positionally conserved, but different in size (Fig. 1B).

3.2. Detection of specific transcripts and determination of the splice junction of THV-2 VTER by RT-PCR

In order to determine the structure of the splice junction and to demonstrate the transcriptional

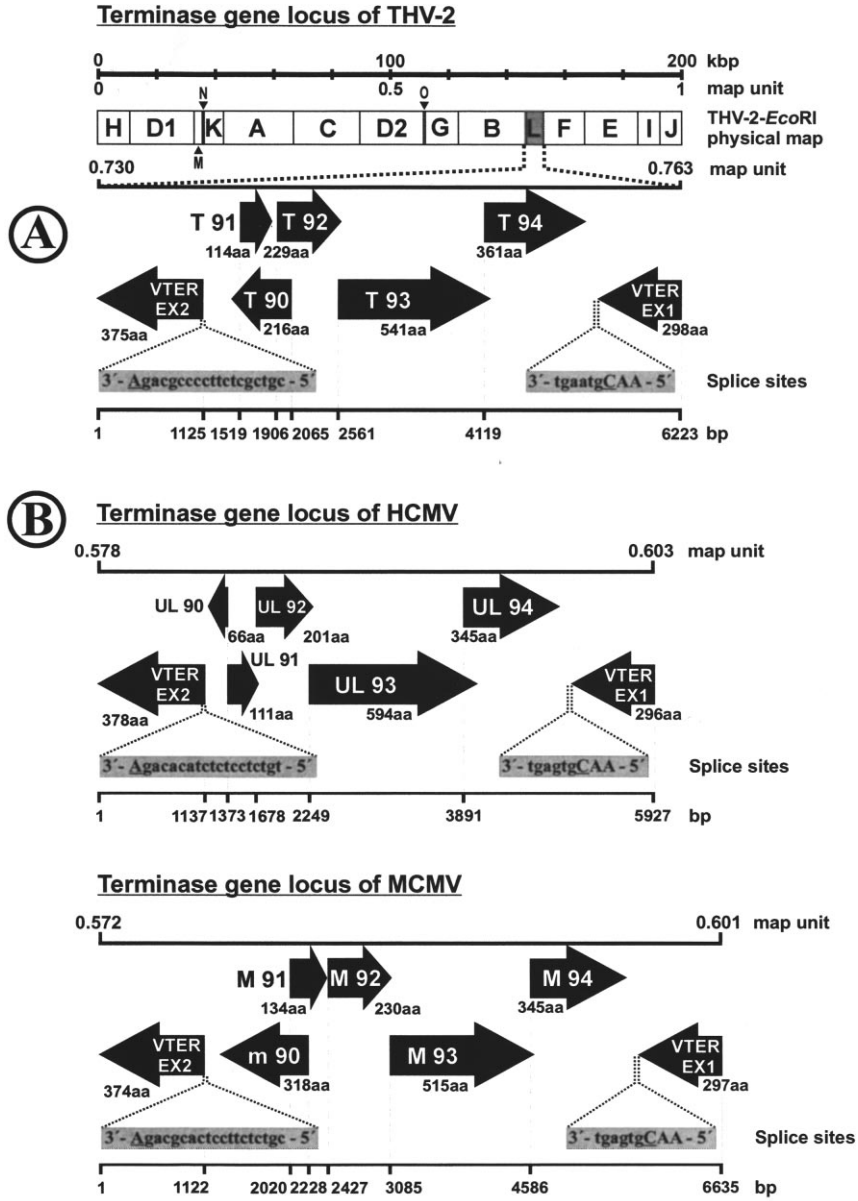

Fig. 1. (A) Diagram of the physical map of the genome of THV-2 for restriction endonuclease *Eco*RI. The positions of the seven ORFs and the sizes of the seven pertinent potential proteins within the grey-shaded region of the DNA sequence of THV-2 between map coordinates 0.730 and 0.763 are illustrated. The positions of the first nucleotide of the start codons of each ORF are given in relation to the last nucleotide of the viral terminase exon 2. Arrows designate the direction of transcription. The donor and acceptor splice sites of the two viral terminase exons are marked by grey-shaded boxes (the capital letters are part of the exons, the small letters belong to the intron; the underlined nucleotides are the two bases that form the splice junction). VTER, viral terminase; Ex1, exon 1; Ex2, exon2; the five ORFs between the two VTER exons are designated with T for THV and numbered according to the homologous ORFs of cytomegaloviruses. (B) Diagrams of the viral terminase gene loci of the Murine cytomegalovirus strain Smith (MCMVS) and the Human herpesvirus 5 strain AD169 (HCMVA). Basepair positions apply to the first nucleotide of the start codons of each ORF and are given in relation to the last nucleotide of the corresponding viral terminase exon 2. Arrows designate the direction of transcription of the genes. The donor and acceptor splice sites of the terminase exons 1 and 2 are marked by grey-shaded boxes.

activity and expression kinetics of the THV-2 VTER gene, viral transcripts were analysed by RT-PCR. The analysis of the region around the splice junction was carried out using two oligonucleotide primers. The first oligonucleotide primer (P # 1) was directed towards the end of exon 1 (starting 155 bp upstream from the putative splice site) and the second (P # 2) towards the start of exon 2 (starting 167 bp downstream from the putative splice site). The experiment was carried out with total cellular RNA from TBF cells that had been infected with THV-2. The RNA was obtained at four different time points post infection, representing the immediate early, early, early late and late stages of the viral replication cycle. The RNA preparation was subjected to DNase I treatment prior to reverse transcription. Control PCRs without reverse transcription treatment were performed to exclude DNA contamination in the RNA preparation. Under these conditions a 323 bp DNA fragment was amplified (Fig. 2A). VTER mRNA was detectable at all four viral replication stages. The expression of VTER under IE conditions is surprising. For this reason a second RT-PCR experiment was performed to determine the real time point at which the VTER mRNA synthesis is detectable. The experiment was carried out as described above with the exception that total RNA of infected TBF cells that was harvested at the 1, 2, 4, and 8 h post infection was used. The results of these studies clearly indicate that the THV-2 VTER mRNA synthesis is between one and two hours post infection detectable (Fig. 2B).

The nucleotide sequence of the 323 bp RT-PCR product was determined and the structure of the splice junction was identified in which the nucleotide sequence of the RT-PCR products were aligned with the DNA nucleotide sequence of the genomic THV-2 *Eco*RI-fragment L. The DNA sequence of the THV-2 splice donor site [5′-AACgtaagt-3′ (the capital letters are the last three nucleotides of exon 1)] was found to be identical with exception of one nucleotide to the splice donor sites of HCMV and MCMV. The sequence of the THV-2 VTER splice acceptor site (5′-cgtcgctcttccccgcagA-3′) is high homologous to those of HCMV and MCMV. In all three viruses the VTER splice acceptor site is characterized by a stretch of pyrimidines that terminates with the

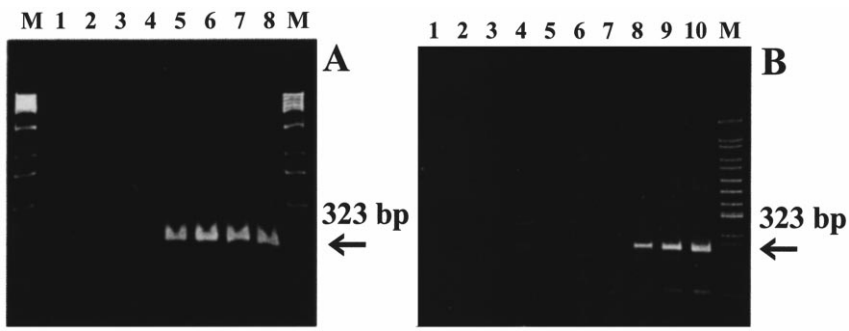

Fig. 2. Polyacrylamid gels showing the results of the RT-PCR experiment to identify the splice site of the viral terminase gene of THV-2. Lanes *M* = 100 bp molecular marker ladder; lanes 5–8 = RT-PCR with total infected cell RNA representing the immediate early (IE, cells infected in presence of cycloheximide, RNA isolated 4 h post infection), early (E, cells infected in presence of cytosine arabinoside, RNA isolated 7 h post infection), early late (EL, RNA isolated 10 h post infection), and late (L, RNA isolated 14 h post infection) stages of infection; lanes 1–4 = controls (same as in lanes 5–8, but without RT step). The number flanking the gel represents the size of the amplified PCR product in base pairs. Following the RT-PCR, the nucleotide sequence of the amplified products were determined in order to identify the splice site of the THV-2 terminase gene. Panel B: Polyacrylamide gel showing the results of the RT-PCR experiment to identify the real time for the synthesis of the THV-2 VTER mRNA after infection. Lanes 1–5 served as internal control (identical to lanes 6–10, but without RT step); lane 6 = RT-PCR with total RNA of uninfected TBF cells; lanes 7–10 = RT-PCR with total RNA of infected TBF cells harvested at different times post infection: 1 h (lane 7), 2 h (lane 8), 4 h (lane 9), and 8 h p.i. (lane 10). Lane M = 100 bp-ladder served as molecular weight marker. The arrows represent the size of the amplified PCR product.

Table 1
Size and homology of the Tupaia herpesvirus terminase (VTER_THV2) in comparison to the viral terminase of different herpesviruses[a]

Terminase (accession number)	Herpesvirus (subfamily)	Size of protein (aa)	Value of amino acid identity with VTER_THV2 (%)	Value of amino acid Similarity with VTER_THV2 (%)
VTER_THV2 (AF228035)	*Tupaia* herpesvirus 2 (ß)	673	100	0
VTER_MCMVS (U68299)	Murine cytomegalovirus (β)	671	68.9	23.8
VTER_HCMVA (P16732)	Human cytomegalovirus (β)	674	66.4	24.9
VTER_HSV6U (P24443)	Human herpesvirus 6 (β)	667	57.8	30.3
VTER_HSV7J (P52462)	Human herpesvirus 7 (β)	663	55.7	32.3
VTER_EBV (P03219)	Epstein–Barr virus (γ)	690	35.1	38.4
VTER_HHV8 (U93872.1)	Human herpesvirus 8 (γ)	687	35.4	38.6
VTER_HSVAL (AF005370)	Alcelaphine herpesvirus 1 (γ)	686	37.2	38.7
VTER_M68 (U97553.1)	Murine herpesvirus 68 (γ)	654	31.4	39.1
VTER_HSVB4 (AF139096.1)	Bovine herpesvirus 4 (γ)	682	34.6	38.2
VTER_HSVSA (Q01020)	Herpesvirus saimiri (γ)	683	35.1	39.5
VTER_HSV11 (P04295)	Human herpesvirus 1 (α)	735	37.2	35.0
VTER_HSV2H (Z86099.1)	Human herpesvirus 2 (α)	734	37.8	34.1
VTER_VZVD (P09294)	Varicella-zoster-virus (α)	747	37.0	34.0
VTER_HSVE1 (P28969)	Equine herpesvirus 1 (α)	734	37.2	36.8
VTER_HSVE4 (AF030027)	Equine herpesvirus 4 (α)	734	36.0	38.5

[a] Percentage values were determined using the CLUSTAL software (PC/GENE release 6.85, Intelligenetics Inc, CA, USA).

characteristic dinucleotide AG and followed by a terminal adenosine, representing the first nucleotide of exon 2 (Fig. 1).

3.3. Analysis of the THV-2 VTER gene locus

The VTER gene locus maps between genome coordinates 0.730 and 0.763 and comprises two exons (map coordinates 0.758–0.763 and 0.730–0.736, respectively). The two exons form an ORF of 2022 nt that encodes a polypeptide of 674 amino acid residues with a predicted molecular mass of 76.9 kDa.

The nucleotide sequence in the vicinity of the ATG start codon of the THV-2 VTER gene (5′-GCTCCATGC-3′) corresponds moderately to the Kozak (1983, 1986) consensus sequence (5′-CC(A/G/T)CCATGG-3′). However, no transcriptional signals were found upstream and downstream of the coding region of the VTER gene.

The deduced amino acid sequence obtained from the assembled nucleotide sequences of the two THV-2 VTER exons was compared to fifteen different VTERs (Table 1). The highest homologies were found between THV-2 and members of the Betaherpesvirinae. The values of amino acid

the Betaherpesvirinae. The values of amino acid sequence homology were found to be 92.7% and 91.3% for MCMV and HCMV, respectively. The deduced amino acid sequence derived from THV-2 VTER exon 1 shows 90% and 89% amino acids homology to the corresponding amino acid sequences of HCMV and MCMV VTER exon 1, respectively. In contrast, the deduced amino acid sequence derived from THV-2 VTER exon 2 reveals homology values of 95.8% and 92.3% to MCMV and HCMV VTER exon 2, respectively.

A detailed comparison of the amino acid sequences of THV-2 VTER and the two counterparts of MCMV and HCMV is shown in Fig. 3. It is evident that the three proteins are extremely high conserved with the exception of the first 150 aa. The splice sites are positionally conserved (between aa 298 and 299 for THV-2 VTER, aa 297 and 298 for MCMV VTER, and aa 296 and 297 for HCMV VTER). An ATP binding site (Walker et al., 1982) was identified within the amino acid sequence of the THV-2 VTER. The Walker Box A (GKT) located at aa positions 219–221 and Walker Box B (DE) at aa positions 311–312 are both positionally exactly conserved in the VTERs of MCMV and HCMV.

any known herpesviral protein. However,

3.4. Analysis of the intron of the THV-2 VTER gene locus

The THV-2 VTER intron maps between genome coordinates 0.736 and 0.758 and contains five potential ORFs, that were termed T90–T94 according to the five collinearly arranged ORFs in the VTER introns of MCMV (m90–M94) and to HCMV (UL91–UL94) (Fig. 1). The lengths of the ORFs and properties of the putative translation products are 648 bp for T90 (216 aa; 22.1 kDa), 342 bp for T91 (114 aa; 11.8 kDa), 687 bp for T92 (229 aa; 25.9 kDa), 1623 bp for T93 (541 aa; 59.7 kDa), and 1083 bp for T94 (361 aa; 39.4 kDa).

The nucleotide sequences, surrounding the putative ATG start codons of the five THV-2 ORFs correspond well (T92) or are slightly divergent (T90, T91, T93, T94) from the consensus sequence of Kozak (1983, 1986). A TATA-box motif (5′-TATAATA-3′) is located between nucleotide positions −311 to −305 upstream of the start codon of T90. A further TATA-box motif (5′-TATTATA-3′) was identified upstream of the T93 start codon between nt −190 and −184. No classical TATA-box motifs were identified for the ORFs T91, T92, and T94. No classical polyadenylation signals could be detected downstream of any of the five THV-2 ORFs. Downstream of the coding regions of T90 and T94 there are short stretches of adenosine nucleotides that could be used as polyadenylation signals.

The deduced amino acid sequences of the THV-2 ORFs T91–T94 were compared to the corresponding counterparts of MCMV, HCMV, HHV-6, Epstein–Barr virus (EBV), and Human herpesvirus 1 (HSV-1) (Table 2). These four ORFs were conserved in all the genomes of the betaherpesviruses MCMV, HCMV, and HHV-6. The highest homology values were found between THV-2 and MCMV (T92, T93, T94) and HCMV (T91). T90 did not show a significant homology to any known herpesviral protein. However, MCMV and HCMV contain a positionally conserved ORF within their VTER introns (Fig. 1). Computer-assisted analysis revealed a putative G-protein coupled receptor signature (SENMAAAALAANRSISI) at aa positions 51–67 in the sequence of T90. THV-2 T94 possesses a histidine residue (aa 234) and six cysteine residues (aa positions 220, 225, 228, 251, 253, and 257) in an arrangement that is reminiscent of that seen in zinc finger or zinc ring proteins (Wing et al., 1996).

3.5. Phylogenetic relatedness

The amino acid sequences of the THV-2 proteins VTER and T91–T94 were compared to the amino acid sequences of the corresponding proteins of Betaherpesvirinae (HCMV, MCMV, HHV-6), Gammaherpesvirinae (EBV), and Alphaherpesvirinae (HSV-1) as shown in Table 2. The highest relatedness was detected between the proteins of THV-2 and the corresponding proteins of the betaherpesviruses. The detected relatedness was not based only on the homology values but also on the positional conservation of the ORF composition within the DNA sequence of the VTER introns.

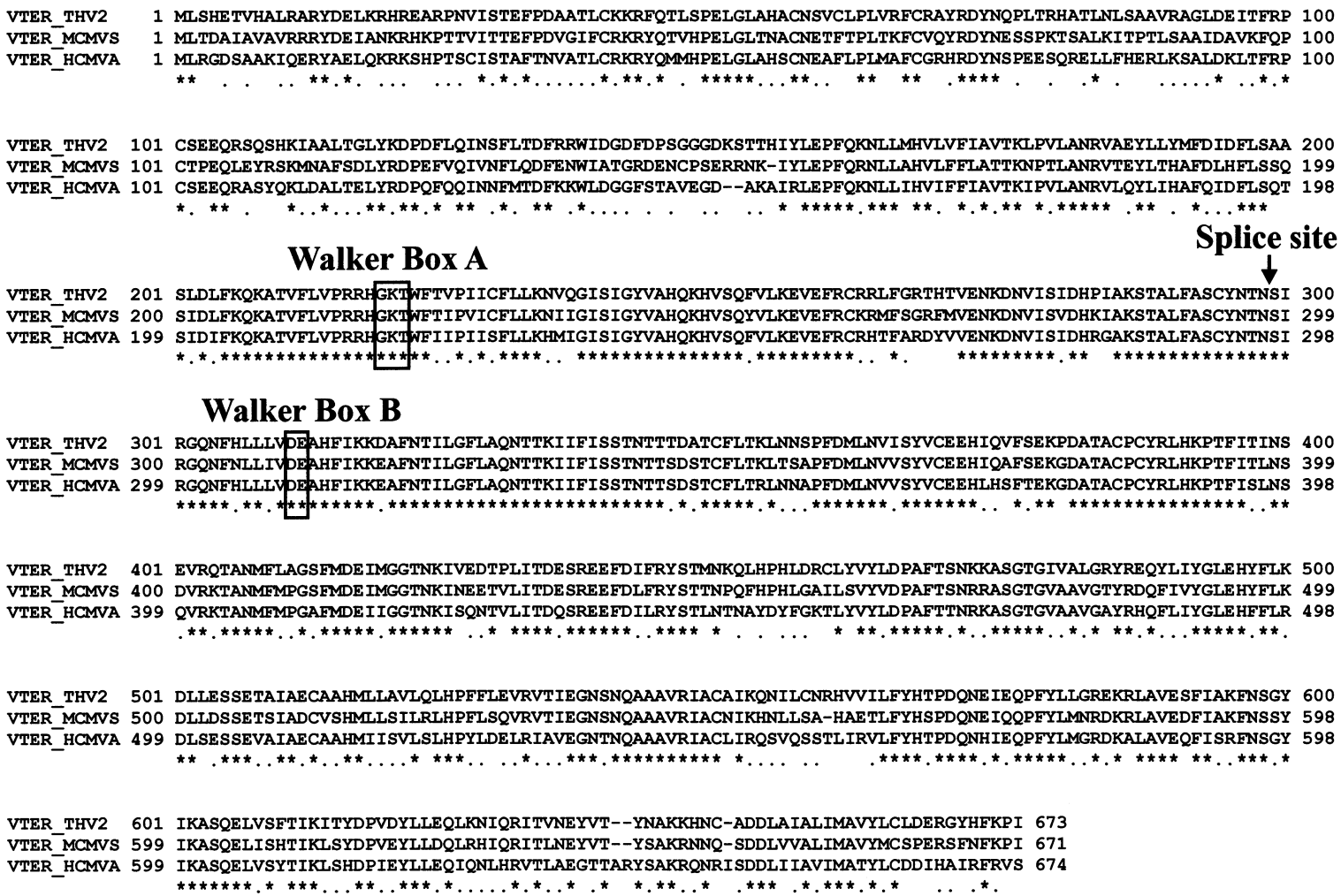

Fig. 3. Alignment of the amino acid sequences of the viral terminases of THV-2, the MCMVS, and the HCMVA. Identical amino acids are denoted by asterisks. The dots indicate the position of well-conserved amino acids. Dashes indicate artificial gaps that have been introduced to achieve maximal amino acid matching. The positions of the splice sites are marked by an arrow. The two parts of the ATP-binding site, Walker Box A and Walker Box B, are framed.

The homology values between THV-2 VTER and the analogous proteins of fifteen different herpesviruses are summarized in Table 1. A dendrogram based on multiple amino acid sequence alignments of these sixteen viral terminases is shown in Fig. 4. These results are a further strong evidence that THV is a member of the subfamily Betaherpesvirinae and closely related to the mammalian cytomegaloviruses.

4. Discussion

Recently we succeeded in characterization of a conserved gene cluster, coding for the DPOL, the gB, a PRTP, and the major DNBI from THV (Bahr et al., 1999; Springfeld et al., 1998). Based on these results THV was classified into the herpesviral subfamily Betaherpesvirinae. In the present study the gene locus of the VTER of THV-2, one of the most highly conserved proteins of herpesviruses, that is suitable for evolutionary studies, was elucidated. VTER is one of several proteins known to be essential for viral DNA cleavage and packaging. In the course of herpesviral DNA replication large head-to-tail concatemers are produced by poorly understood processes (Bataille and Epstein, 1995; Severini et al., 1994, 1996). The precise mechanism of the herpesviral DNA cleavage and packaging process, in which monomeric genomes are released from concatemeric DNA molecules by cleavage at specific target sites (Deiss et al., 1986), is still unclear. However in the case of dsDNA bacteriophages, that share common features with herpesviruses concerning DNA replication and maturation, the VTER is a key component of the DNA cleavage and packaging machinery with at least two distinct roles, cleavage and the energy requiring step of translocation of DNA (Black, 1989). In bacteriophage T4 the terminase complex is composed of two subunits, the large catalytic subunit gp17 and the small subunit gp16 (Kuebler and Rao, 1998; Lin et al., 1997). The catalytic subunit (gp17), shares limited homology with the HSV-1 UL15 protein (Davison, 1992), that is assumed to be the catalytic subunit of the HSV-1 terminase protein complex (Yu and Weller, 1998; Baines et al., 1994) and that is homologous to the THV-2 VTER. Furthermore, it is known that HCMV PRTP possesses the activity of a packaging motif binding protein and a specific nuclease (Bogner et al., 1998). The resistance of Human herpesvirus 5 to benzimidazole ribonucleosides (prevention of maturation of concatemeric DNA to unit genome length) was mapped to the VTER and PRTP ORFs suggesting that these two proteins may interact (Krosky et al., 1998; Underwood et al., 1998).

Table 2
Degree of homology found between the five potential proteins VTER, T91, T92, T93 and T94 of *Tupaia* herpesvirus (THV) to the corresponding proteins of Human cytomegalovirus (HCMV; X17403), Murine cytomegalovirus (MCMV; U68299), human herpesvirus 6 (HHV6; X83413.1), Epstein–Barr virus (EBV; V01555.1) and herpes simplex virus (HSV-1; X14112.1)[a]

THV Proteins	Homologous protein									
	HCMV		MCMV		HHV6		EBV		HSV-1	
	%	Protein	%	Protein	%	Protein	%	Protein	%	Protein
VTER	91.3	VTER	92.7	VTER	88.1	VTER	73.5	VTER	72.2	VTER
T91	80.7	UL91	45.6	M91	55.3	U62	n.h.[b]	n.h.	n.h.	n.h.
T92	77.3	UL92	85.2	M92	78.9	U63	67.4	BDLF4	n.h.	n.h.
T93	56.1	UL93	65.9	M93	65.0	U64	55.8	BGLF1	50.5	UL17
T94	68.6	UL94	73.5	M94	69.2	U65	57.6	BGLF2	54.4	UL16

[a] Percentage values were determined using the CLUSTAL software (PC/GENE release 6.85, Intelligenetics Inc, CA, USA) and homology is the sum of the percentange values for identical and similar amino acids between the THV-2 proteins and five corresponding proteins of the five other herpesviruses.

[b] n.h. – no homologous protein.

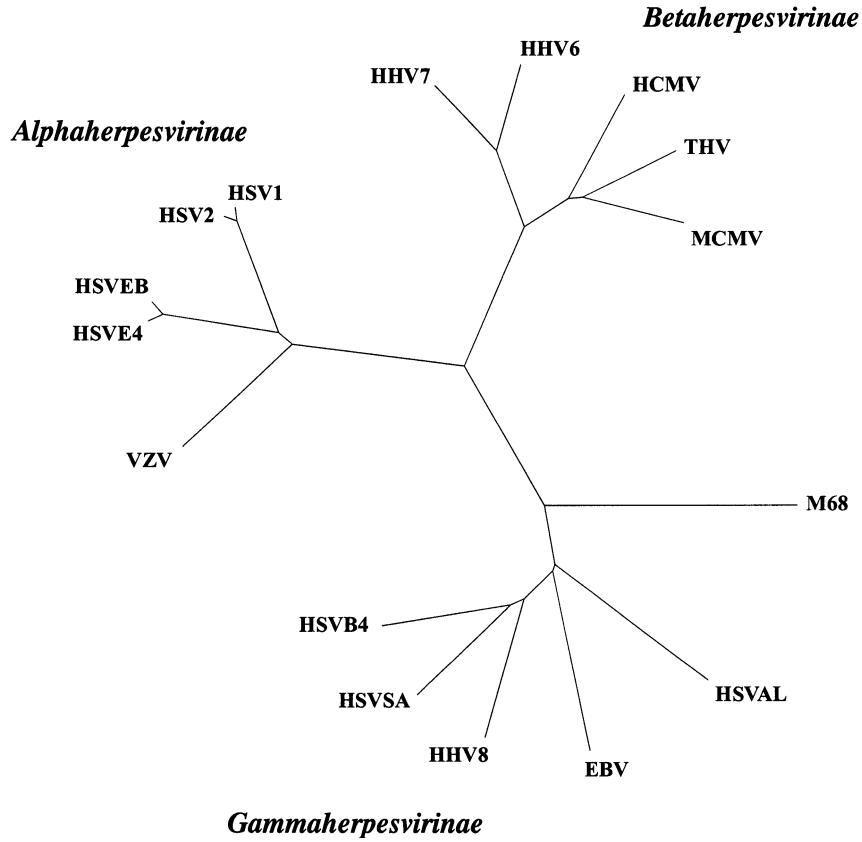

Fig. 4. Phylogenetic tree based on the alignment of the viral terminase amino acid sequence of 16 herpesviruses including EBV (Epstein–Barr-virus strain 95-8), HCMV (Human herpesvirus 5 strain AD169), HHV6 (Human herpesvirus 6 strain Uganda-1102), HHV7 (Human herpesvirus 7 strain JI), HHV8 (Human herpesvirus 8), HSVAL (Alcelaphine herpesvirus 1 strain C500), HSV1 (Human herpesvirus 1 strain 17), HSV2 (Human herpesvirus 2 strain HG52), HSVB4 (Bovine herpesvirus 4 strain 66-p-347), HSVEB (Equine herpesvirus 1 strain AB4P), HSVE4 (Equine herpesvirus 4), HSVSA (Herpesvirus saimiri strain 11), M68 (Murine herpesvirus 68), MCMV (Murine cytomegalovirus strain Smith), THV (Tupaia herpesvirus strain 2), VZV (Varicella-zoster-virus strain Dumas). The three main branches of the tree represent the herpesviral subfamilies Alpha-, Beta-, and Gammaherpesvirinae.

The THV-2 VTER possesses a putative ATP binding site as has been demonstrated for gp17 and other phage terminases (Backhaus, 1985; Black, 1989; Casjens et al., 1987). The herpesviral DNA cleavage and packaging process takes place in the nucleus of infected cells, so it is necessary for VTER to migrate into the nucleus. However, no nuclear localization signal was identified in the aa sequence of the viral terminase of THV-2.

The intron of the THV-2 VTER gene locus contains the five potential ORFs T90–T94. For T90 no homologous protein was found and no function could be assigned to this protein. This is also true for T91 and T92 and for the corresponding homologous proteins in other herpesviruses. T93 shows homology to HSV-1 UL17, one of the seven HSV-1 genes necessary for DNA cleavage and packaging. It is assumed that UL17 is associated with the virion tegument (Salmon et al., 1998) and is required correct targeting of major and minor capsid proteins to specific nuclear compartments during the DNA cleavage and packaging process (Taus et al., 1998). T94 is homologous to HCMV UL94 and HSV-1 UL16. UL16 seems

to play a role in capsid maturation including DNA packaging and cleavage (Oshima et al., 1998; Nalwanga et al., 1996). In a similar context UL94 of HCMV was found to be associated with the capsid/tegument fraction of virion particles (Wing et al., 1996). In addition, T94 shows conservation of a potential zinc binding motif compared to HCMV UL94, underlining the functionality of T94. A remarkable feature of the six THV-2 ORFs analysed in this study is the absence of classical transcription start and termination signals. However, transcription of these genes could be achieved by specific transcription regulatory mechanisms as has been determined for the HCMV UL94 late promotor (Wing et al., 1996) or probable polycistronic mRNAs as shown for HCMV UL93 through UL99 (Wing and Huang, 1995).

The genetic-structural comparison of the THV-2 VTER gene locus with the corresponding genome regions in other herpesviruses confirms the classification of THV as a member of the subfamily Betaherpesvirinae with highest relatedness to MCMV and HCMV. Gene arrangement and transcriptional directions are exactly collinear between THV-2, MCMV, and HCMV including T90, UL90, and m90. THV, MCMV, and HCMV seem to have similar evolutionary distances from each other. This finding indicates that the present classification of MCMV and HCMV into the two genera *Muro-* and *Cytomegalovirus*, which is mainly based on the overall genomic structure in regard of presence and arrangement of large repetitive elements, should no longer be maintained. The comparison of viral genes on the sequence level supplies excellent data for determination of the stage of evolutionary relatedness of virus species. Therefore, it is recommended to classify all cytomegalovirus species including the THV into one single genus within the Bataherpesvirinae subfamily.

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