FEBS Letters 580 (2006) 4473–4478

Cyprinid herpes virus-3 (CyHV-3) bears genes of genetically distant large DNA viruses

Maya Ilouze[a], Arnon Dishon[a], Tamar Kahan[b], Moshe Kotler[a,*]

[a] *Department of Pathology, The Hebrew University-Hadassah Medical School, Jerusalem 91120, Israel*
[b] *Bioinformatics Unit, The Hebrew University-Hadassah Medical School, Jerusalem 91120, Israel*

Received 9 April 2006; revised 29 June 2006; accepted 6 July 2006

Available online 14 July 2006

Edited by Hans-Dieter Klenk

Abstract A large DNA virus, designated koi herpes virus (KHV), carp interstitial nephritis gill necrosis virus (CNGV) and Cyprinid herpes virus-3 (CyHV-3), causes massive mortality of carp. Morphologically, the virus resembles herpes viruses, but it contains a genome of ca 295 kbp, larger than that of any *Herpesviridae* member. Interestingly, three CyHV-3 genes, thymidylate monophosphate kinase (TmpK), ribonucleotide reductase and thymidine kinase, which are involved in deoxynucleotide tri-phosphate synthesis, resemble those of pox viruses. In addition to the TmpK gene, which is nonexistent in the genome of herpes viruses, CyHV-3 contains a B22R-like gene, exclusively expressed by pox viruses. These results raise questions on the phylogenic origin of CyHV-3.

Keywords: CyHV-3; CNGV; KHV; Genetic divergence; Pox-like genes

1. Introduction

A virus causing a worldwide lethal disease in carp and koi fish was first reported in 1998 by Ariav et al. [1] and Bretzinger et al. [2]. The virus was isolated by Hedrick et al. [3] and then independently by Perelberg et al. [4]. The vast economic losses caused by this virus led to a flurry of research activity around the world, resulting in rapid accumulation of comprehensive data [5–7].

The agent of the carp disease is an enveloped virus with an icosahedral electron-dense core of 100–110 nm surrounded by a tegument-like structure, causing it to resemble a herpes virus [3,8]. The genome of the isolated virus comprises linear double-stranded DNA (dsDNA) of ca 277–295 kbp [8], similar to that of Cyprinid herpes virus-1 (CyHV-1) [9], indicating that the genomes of this virus and CyHV-1 are larger than those of other *Herpesviridae* members. The genome of this virus contains highly divergent DNA sequences encoding polypeptides, which resemble those of several dsDNA viruses, namely herpes, pox, irido and other large DNA viruses [8,9].

The unique characteristics of the virus have led to its identification by three different names: koi herpes virus (KHV) [3], carp interstitial nephritis and gill necrosis virus (CNGV) [10,11] and Cyprinid herpes virus-3 (CyHV-3) [9]. Recently, Waltzek et al. [9] showed that the DNA sequences of the major capsid protein (MCP, Accession no. #AY939864), intercapsomeric triplex (ITP, Accession no. #AY939859), DNA polymerase (DNAP, Accession no. #AY939862), DNA helicase (HLC, Accession no. #AY939857) genes and an unidentified open reading frame of CNGV (ORF, Accession no. #AY208988) are similar to their counterpart genes of Cyprinid herpes virus-1 (CyHV-1) and Cyprinid herpes virus-2 (CyHV-2). These results suggest that CNGV(KHV) and the other CyHVs are members of a unique viral group, and CNGV(KHV) was thus designated CyHV-3 [9]. Yet, the International Committee on Taxonomy of Viruses (ICTV) has not named this virus, but since CNGV(KHV) and CyHVs have some common characteristics, such as morphology, a large DNA genome and several similar genes at the DNA level, in this report we will use the term CyHV-3 until an official name is assigned to this virus by the ICTV.

Here, we demonstrate that some of the CyHV-3 protein sequences are not found in herpes viruses. The B22R-like gene is especially interesting, since it is expressed exclusively by pox viruses. Other CyHV-3 genes, although present in herpes viruses, show a higher degree of similarity to pox viruses. These findings should be helpful in understanding the evolution of CyHV-3, as well as CyHV-1 and CyHV-2, which were identified previously as KHV, herpesvirus cyprini and herpesviral haematopoietic necrosis virus, respectively [3,12,13], thus aiding in their classification.

2. Materials and methods

2.1. Cell culture and virus

Cultures of koi fin cells (KFC) were prepared as previously described by Ronen et al. [11], using the methods of Hasegawa et al. [14] and Neukirch et al. [15]. CyHV-3 isolated from kidneys of sick fish was propagated in KFC cultures, as described previously [8,11].

2.2. Virus purification and DNA extraction

The culture medium harvested from infected KFC was collected and cleared of cells and cell debris by centrifugation for 10 min at $10000 \times g$. The virus was then pelleted by centrifugation in a Beckman Ti-60 rotor for 50 min at $100000 \times g$. Pellets were suspended in PBS,

*Corresponding author. Fax: +972 2 6758190.
E-mail address: mkotler@cc.huji.ac.il (M. Kotler).

Abbreviations: CNGV, carp interstitial nephritis gill necrosis virus; KHV, koi herpes virus; CyHV, Cyprinid herpes virus; IcHV-1, Ictalurid herpes virus 1; RaHV-1, Ranid herpes virus 1; SalHV-1, Salmonid herpes virus 1; AngHV-1, Anguillid herpes virus 1; TmpK, thymidylate monophosphate kinase; RNR, ribonucleotide reductase; TK, thymidine kinase; MCP, major capsid protein; ITP, intercapsomeric triplex; DNAP, DNA polymerase; HLC, helicase; KFC, koi fin cells

0014-5793/$32.00
doi:10.1016/j.febslet.2006.07.013

loaded on a 15–65% (w/v) sucrose gradient prepared in PBS, and centrifuged for 60 min at 110 000 × *g* in a Beckman SW28 rotor. Bands were visualized, aspirated from tubes, diluted 10-fold in PBS and repelleted. Purified viral pellets were suspended and the viral DNA was purified as described by Hutoran et al. [8].

2.3. DNA sequencing and BLAST analysis

Sequencing was performed by "DNA walking" using internal primers derived from previously sequenced fragments (see supplementary data, Appendix 5), with purified viral DNA as templates, and carried out by the dideoxynucleotide terminator cycle sequencing method employing a Prism BigDye Ready Reaction Terminator cycle sequencing kit (PE Applied Biosystems). Reaction and cycling conditions were chosen according to the manufacturer's protocol. Sequencing reactions were run on an ABI 3700 DNA analyzer (PE Applied Biosystems, performed at the Center for Genomic Technologies, The Hebrew University) and the resulting sequences were analyzed by BLAST [16].

2.4. Multiple alignments

Multiple alignments were carried out using "pileup" with default parameters, with the consensus displayed using "prettybox" (GCG – Genetics Computer Group – Wisconsin Package Version 10, Madison, WI).

3. Results

3.1. Partial CyHV-3 genome organization

Analysis by BLAST of the publicly available ~20% of the viral genome was in agreement with our previous findings, showing that CyHV-3-DNA is divergent from all other known viruses [8]. Analysis of all the viral genome sequences available in the databases shows that there are no fragments longer than 45 bp which are similar to the sequences in all other viral genomes except those of CyHV-1 and CyHV-2 [9]. Many of these small fragments are found in genomic sequences of the *Herpesviridae*, *Adenoviridae*, *Baculoviridae* and *Poxviridae* families [8].

Fig. 1 shows that, as in many other organisms, both strands of the CyHV-3 genome encode for viral genes. One example is fragment C, which reveals that RNR and TmpK are two genes in juxtaposition and are transcribed from the positive and negative DNA strands, respectively.

By using the ORF finder program (http://www.ncbi.nlm.nih.gov/gorf/gorf.html), we found that seven polypeptides, namely ORF1 (Accession no. #BAD18063), ORF2 (Accession no. #BAD18064), ORF3 (Accession no. #BAD18065), ORF4 (Accession no. #BAD18066), glycoprotein (Accession no. #AAY67836), ORF6 (Accession no. #AAW47586) and ORF7 (Accession no. #AAZ23014), do not resemble any known viral proteins. On the other hand, CyHV-3 bears at least four genes that encode proteins similar to those expressed by pox viruses: thymidylate monophosphate kinase (TmpK), ribonucleotide reductase (RNR) and thymidine kinase (TK), which are involved in the synthesis of dNTPs required for DNA synthesis, and for B22R-like gene (Fig. 1).

3.2. Multiple amino acid sequence alignments of CyHV-3 and other large DNA viruses

Fig. 2 shows alignments of three CyHV-3 gene products with homologous proteins expressed by *Poxviridae* members, and of the TmpK gene product, which also resembles *Iridoviridae* and *Nimaviridae* protein sequences. The identity and similarity of these four proteins are summarized in Table 1, which shows that the similarity values of these proteins are in the range of 29–55% and 45–72%, respectively. The similarity between pox viruses and CyHV-3 is especially interesting as the former are morphologically distinguishable from CyHV-3 and unlike CyHV-3, propagate entirely in the host cell cytoplasm. Table 1 and Fig. 2 clearly show that the TK, RNR and TmpK genes are primarily similar to genes encoded by pox viruses, although

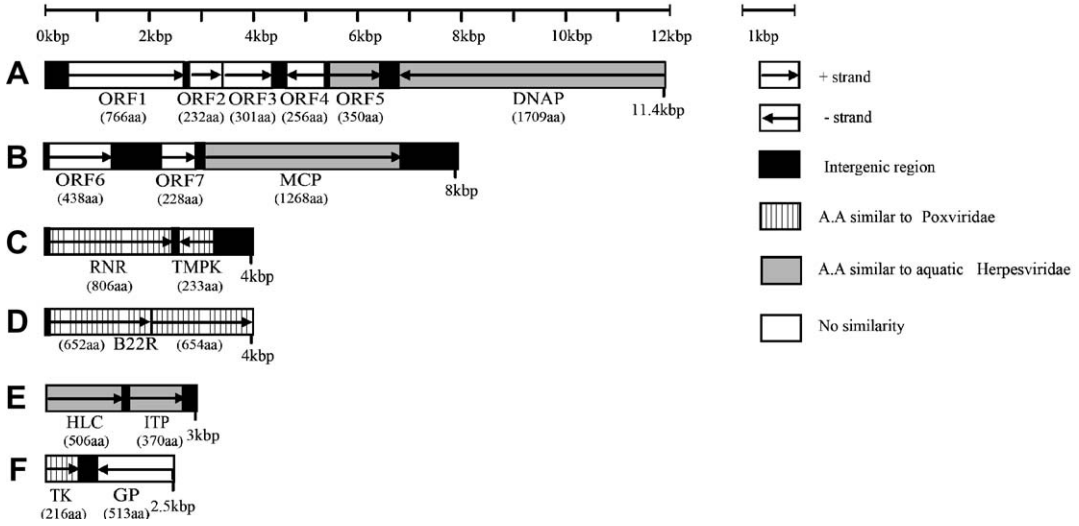

Fig. 1. Partial CyHV-3 genome organization. The CyHV-3 DNA fragments (A–E) were sequenced and subsequently analyzed using the ORF finder program of NCBI (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) and employing open reading frames. *Fragment A*: ORF1 – hypothetical protein (Accession no. #BAD18063), ORF2 – membrane protein (Accession no. #BAD18064), ORF3 – membrane protein (Accession no. #BAD18065), ORF4 – major envelope protein (Accession no. #BAD18066), ORF5 – hypothetical protein (Accession no. #BAD18067), DNA Polymerase (DNAP, Accession no. #AAX53082); *Fragment B*: ORF6 – hypothetical protein (Accession no. #AAW47586), ORF7 – hypothetical protein (Accession no. #AAZ23014), major capsid protein (MCP, Accession no. #AAY41899); *Fragment C*: ribonucleotide reductase – large subunit (RNR, Accession no. #AAY99446), thymidylate monophosphate kinase (TmpK, Accession no. #AAZ23013); *Fragment D*: B22R-like gene (B22R, Accession no. #AY661550); *Fragment E*: DNA helicase (HLC, Accession no. #AAX53076), intercapsomeric triplex protein (ITP, Accession no. #AAX53079); *Fragment F*: thymidine kinase (TK, Accession no. #CAD59379); glycoprotein (GP, Accession no. #AAY67836).

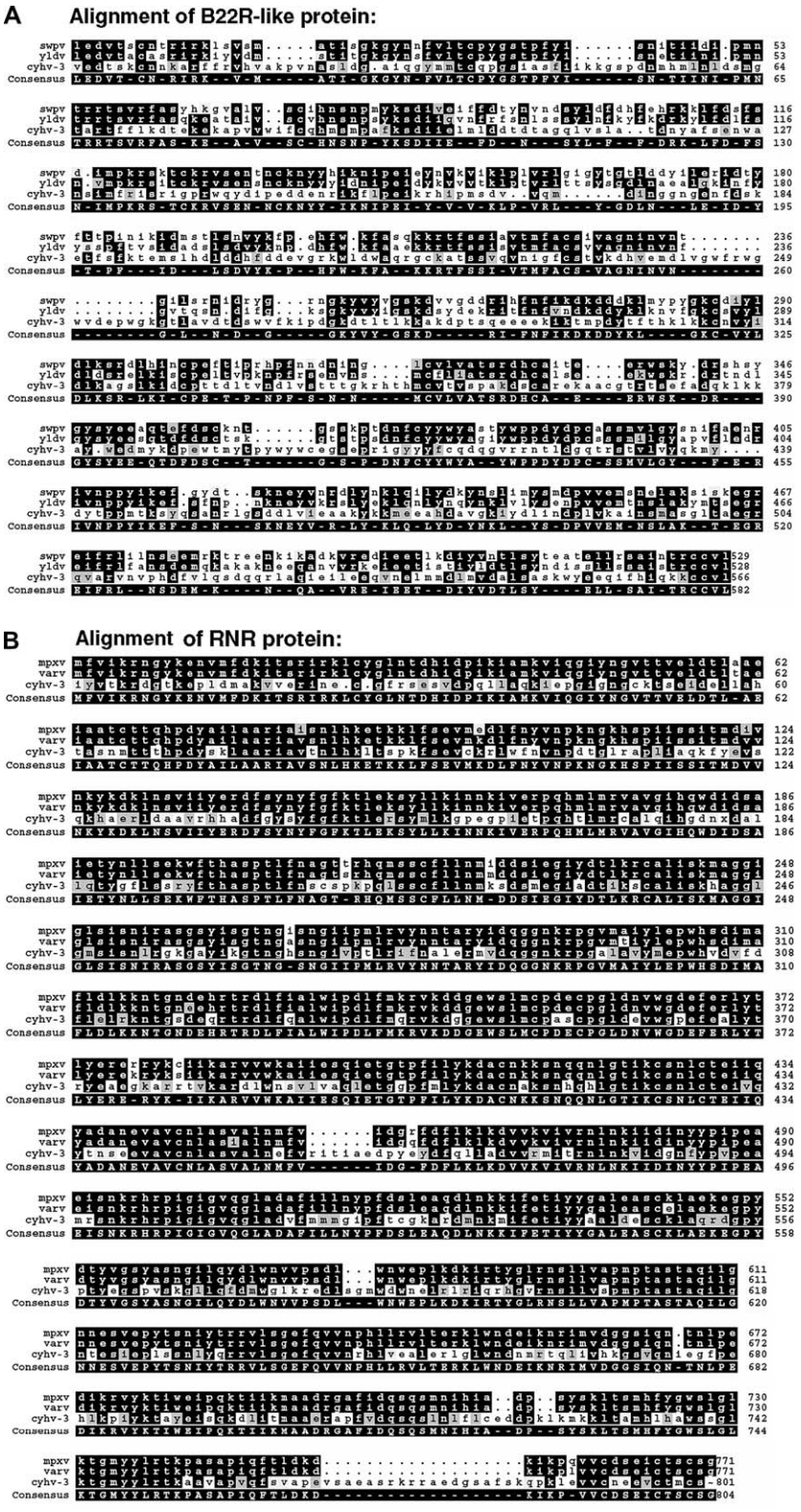

Fig. 2. Alignment of the CyHV-3 protein sequences B22R-like gene (A), RNR (B), TK (C) and TmpK (D). The CyHV-3 TK (Accession no. #AJ535112), B22R-like gene (Accession no. #AY661550), RNR (Accession no. #AY786308) and TmpK (Accession no. #DQ118125) translated nucleotide sequences were analyzed using BLASTX against the viral database (http://www.ncbi.nlm.nih.gov/BLAST). The sequences included in alignments A–C are those with the highest BLAST scores. In D, the sequences included are those with the highest BLAST score derived from three different virus families. The VACV (Vaccinia virus), CPXV (Cowpox Virus), CNPV (Canarypox virus), MPXV (Monkeypox virus), SWPV (Swinepox virus), YLDV (Yaba-like disease virus), VARV (Variola virus) – all from the *Poxviridae* family; WSSV (Shrimp white spot syndrome virus) – *Nimaviridae* family; CIV (Chilo iridescent virus) – *Iridoviridae* family. Appendices 1–4 include all significant BLAST results of these four protein sequences.

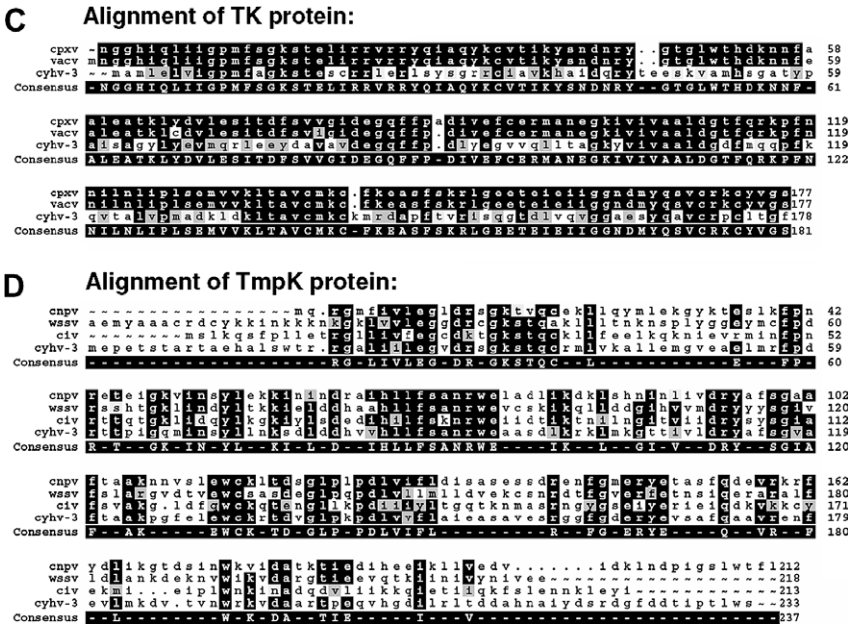

Fig. 2 (*continued*)

Table 1
Comparison of CyHV-3 to representatives of Pox viruses and other large DNA viruses

Gene and Accession no. #	Family	Genus	Acc#	Identity (%)	Similarity (%)	Gaps (%)
B22R like gene (Accession no. AY661550)	*Poxviridae*[1]	Fowlpox virus	Accession no. CAE52648	78/262 (29%)[a]	133/262 (50%)[a]	8/262 (3%)[a]
Thymidylate monophosphate kinase (Accession no. DQ118125)	*Poxviridae*[2]	Canarypox virus	Accession no. NP_955193	106/216 (49%)	143/216 (66%)	8/216 (3%)
	Nimaviridae	Shrimp white spot syndrome virus	Accession no. AAK77840TK-dTMP	82/191 (42%)	117/191 (61%)	1/191 (0%)
	Iridoviridae	Chilo iridescent virus	Accession no. AAK82112	72/176 (40%)	117/176 (66%)	3/176 (1%)
	Asfarviridae	African swine fever virus	Accession no. CAA79604	57/192 (29%)	89/192 (46%)	8/192 (4%)
Ribonucleotide reductase (Accession no. AY786308)	*Poxviridae*[3]	Monkeypox virus	Accession no. AAU01269	447/803 (55%)	580/803 (72%)	35/803 (4%)
	Baculoviridae[3]	*Spodoptera litura* nucleopolyhedrovirus	Accession no. AAL01709	415/763 (54%)	555/763 (72%)	13/763 (1%)
	Phycodnaviridae[3]	Feldmannia irregularis virus a	Accession no. AAR26844	394/765 (51%)	529/765 (69%)	12/765 (1%)
	Mimivirus	*Acanthamoeba polyphaga* mimivirus	Accession no. YP_142667	346/733 (47%)	489/733 (66%)	12/733 (1%)
	Nimaviridae	Shrimp white spot syndrome virus	Accession no. AAL89096	379/845 (44%)	538/845 (63%)	49/845 (5%)
	Asfarviridae	African swine fever virus	Accession no. P42491	285/737 (38%)	415/737 (56%)	64/737 (8%)
	Herpesviridae[3]	Ostreid herpesvirus 1	Accession no. YP_024594	263/741 (35%)	415/741 (56%)	58/741 (7%)
Thymidine kinase (Accession no. AJ535112)	*Poxviridae*[4]	Cowpox virus	Accession no. AAF21104	71/171 (41%)	114/171 (66%)	3/171 (1%)
	Mimivirus	*Acanthamoeba polyphaga* mimivirus	Accession no. YP_142612	60/181 (33%)	102/181 (56%)	14/181 (7%)
	Nimaviridae	Shrimp white spot syndrome virus	Accession no. AAG40728	69/178 (38%)	100/178 (56%)	8/178 (4%)
	Asfarviridae	African swine fever virus	Accession no. AAQ07945	52/177 (29%)	81/177 (45%)	10/177 (5%)

Four CyHV-3 DNA sequences were analyzed using translating BLAST (http://www.ncbi.nlm.nih.gov/BLAST). The search was carried out against the Viruses subclass of the NR database. Representatives from different virus families are presented in the table. Identity (%) is the number of amino acids with identity/total number of amino acids of the protein; Similarity (%) is the number of conserved amino acids/total number of amino acids; Gaps (%) are numbers of gaps/total number of amino acids. 1–4: See Appendices for the full list of BLAST results.
[a]The assessed fragment does not represent the sequences of complete protein.

they have significant similarity to other large DNA viruses, such as members of *Nimaviridae*, *Iridoviridae*, *Asfaviridae*, *Baculoviridae*, *Herpesviridae* and others (see Appendices 1–4). Importantly, TmpK and B22R-like genes are not encoded by any *Herpesviridae* member [17,18], while the B22R-like gene is exclusively expressed in pox viruses. All our efforts to construct a phylogenic tree based on the available sequences in the databases have failed so far. It is impossible to relate the CyHV-3 genomic sequence to any other known viral genomes, and even the amino acid sequences of the available ORFs are very different from those of other organisms.

3.3. CyHV-3 bears cellular homologous genes

The TK gene of CyHV-3 not only resembles other viral homologous proteins, but also the equivalent gene product of *Trypanosoma brucei* – a protozoan organism [23]. TK is not the sole CyHV-3 gene resembling its eukaryotic homolog, as RNR and TmpK are also similar to their cellular counterparts. This may indicate that CyHV-3, like other large DNA viruses, bears protein sequences similar to those expressed by the host cellular genome.

4. Discussion

CyHV-3 fragments A, B and E (Fig. 1) encode DNAP, MCP, HLC, ITP and hypothetical protein ORF5, which are similar to those of CyHV-1, CyHV-2 and other aquatic *Herpesviridae* members, such as Ictalurid herpes virus 1 (IcHV-1) [20], Ranid herpes virus 1 (RaHV-1) [21], Salmonid herpes virus 1 (SalHV-1) [22] and Anguillid herpes virus 1 (AngHV-1) [19]. These results suggest that CyHV-1, CyHV-2 and CyHV-3 and the other aquatic herpes like viruses could be classified as one group, although we still do not know whether the CyHVs bear the B22R-like gene, TK, TmpK and RNR, and if so, whether they are similar to those of CyHV-3. Moreover, some of the viral ORFs show no similarity to any known proteins encoded by other organisms. At present, the size of the genome [11], its prominent diversity [8] and the presence of genes resembling pox and other DNA viruses in the CyHV-3 DNA make analysis of evolutionary origin and classification of CyHV-3 and the other CyHVs difficult. Additional genetic and biological data are vital if these viruses are to be classified.

Among the aquatic viruses, only the genome of IcHV-1 has been completely sequenced. Because this viral genome is phylogenetically distant from the "classical" α, β and γ mammalian and avian herpes viruses, it was suggested to include IcHV-1 in a separate group designated "*Alloherpesviridae*" [24]. Because IcHV-1 is similar to the other aquatic herpes like viruses [9], it can be expected that all these viruses would be grouped together. However, here we demonstrated that IcHV-1 does not encode the pox-like genes of CyHV-3: TmpK, RNR and B22R-like gene, while its TK protein is distinguished from its counterpart in CyHV-3, suggesting that the phylogenic distance between IcHV-1 and CyHV-3 is sufficient to warrant inclusion in a separate group.

Recently, Rijsewijk and his co-workers demonstrated that CyHV-3 and AngHV-1 are closely related according to their DNA polymerase protein sequences. Although the amino acid sequences of these two viruses are similar to those of RaHV-1 and IcHV-1, it was proposed that they be included in a separate group [25]. The enlarged genomic sizes of the CyHVs could result from horizontal gene transfer into the genomes of these aquatic herpes like viruses. Alternatively, "small" aquatic herpes viruses (IcHV-1) could have degenerated from the CyHVs, by losing some of their genes during evolution.

The CyHV-3 genome is composed of evolutionarily-distant elements, which are derived from pox, herpes, irido and baculo viruses (Figs. 1 and 2) [8]. Genes from phylogenetically distant viruses could have been incorporated into the CyHV-3 genome by recombination between viruses infecting the same cell. However, this seems improbable in this case, since herpes and pox viruses propagate in different cell compartments. It is also possible that CyHV-3 and the other CyHVs developed independently from a distant family of large DNA viruses, acquiring *Herpesviridae* morphology. Alternatively, CyHV-3 may represent an ancestor of an old viral family, from which several viral groups such as herpes, pox and irido viruses could have originated. Constructing phylogenic trees could be helpful in resolving this problem. However, the diversity of these genes from all other sequences available in the databases does not allow us to assign the CyHV-3 sequences to a conclusive site, suggesting that its phylogeny is too distant to warrant inclusion in a separate family.

The available data demonstrate that TmpK and B22R-like gene are two genes not expressed by aquatic herpes like viruses, and that the CyHV-3-TK gene cannot be found in any fish herpes viruses [19]. The similarity of CyHV-3 TK to the homologous pox virus enzymes explains why we and others failed to inhibit CyHV-3 propagation with Acyclovir and Gancyclovir [19]. Completing the sequencing of the CyHV-3 and CyHVs will clarify whether they should be grouped together, and whether the genomic information justifies classifying them as herpes viruses. Should this be the decision, the definition of *Herpesviridae* must be expanded to include viruses with a genome of ca 300 kbp [8,9,11]. Moreover, CyHV-3 RNR and TmpK are also similar to their cellular counterparts, but this similarity lies beyond the scope of this discussion.

Importantly, some of the CyHV-3 protein sequences are similar to those of viruses that constitute sole representatives of their family, such as the African swine fever virus of the *Asfaviridae*. Whatever the phylogenic process, it is clear that CyHV-3, and probably the other CyHVs, contain genes which have never been described in mammalian, avian and reptile herpes viruses, e.g., TmpK and B22R-like gene. Moreover, about 80% of the CyHV-3 sequences deposited in the databases are not similar to any known sequence. Intensive phylogenic studies and additional biological data are needed to determine whether to include all aquatic herpes viruses together with CyHVs in a single unique group under the *Herpesviridae* umbrella.

Acknowledgements: We thank Mrs. S. Amir and C. Balshayi for editing this manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2006.07.013.

References

[1] Ariav, R., Tinman, S., Paperna, I. and Bejerano, I. (1998) Presented at the EAFP 9th International Conference, Rhodes, Greece.

[2] Bretzinger, A., Fischer-Scherl, T., Oumouma, R., Hoffmann, R. and Truyen, U. (1999) Mass mortalities in koi, *Cyprinus carpio*, associated with gill and skin disease. Bull. Eur. Assoc. Fish Pathol. 19, 182–185.

[3] Hedrick, R.P., Gilad, O., Yun, S., Spangenberg, J.V., Marty, G.D., Nordhausen, R.W., Kebus, M.J., Bercovier, H. and Eldar, A. (2000) A herpesvirus associated with mass mortality of juvenile and adult koi, a strain of common carp. J. Aquat. Anim. Health 12, 44–55.

[4] Perelberg, A., Smirnov, M., Hutoran, M., Diamant, A., Bejerano, I. and Kotler, M. (2003) Epidemiological description of a new viral disease afflicting cultured *Cyprinus carpio* in Israel. Isr. J. Aquaculture-Bamidgeh 55, 5–12.

[5] Ilouze, M., Dishon, A. and Kotler, M. (2006) Characterization of a novel virus causing a lethal disease in carp and koi. Microbiol. Mol. Biol. Rev. 70, 147–156.

[6] Pokorova, D., Vesely, T., Piackova, V., Reschova, S. and Hulova, J. (2005) Current knowledge on koi herpesvirus (KHV): a review. Vet. Med. Czech. 50, 139–147.

[7] Haenen, O.L.M., Way, K., Bergmann, S.M. and Ariel, E. (2004) The emergence of koi herpesvirus and its significance to European aquaculture. Bull. Eur. Assoc. Fish Pathol. 24, 293–307.

[8] Hutoran, M., Ronen, A., Perelberg, A., Ilouze, M., Dishon, A., Bejerano, I., Chen, N. and Kotler, M. (2005) Description of an as yet unclassified DNA virus from diseased *Cyprinus carpio* species. J. Virol. 79, 1983–1991.

[9] Waltzek, T.B., Kelley, G.O., Stone, D.M., Way, K., Hanson, L., Fukuda, H., Hirono, I., Aoki, T., Davison, A.J. and Hedrick, R.P. (2005) Koi herpesvirus represents a third cyprinid herpesvirus (CyHV-3) in the family *Herpesviridae*. J. Gen. Virol. 86, 1659–1667.

[10] Pikarsky, E., Ronen, A., Abramowitz, J., Levavi-Sivan, B., Hutoran, M., Shapira, Y., Steinitz, M., Perelberg, A., Soffer, D. and Kotler, M. (2004) Pathogenesis of acute viral disease induced in fish by carp interstitial nephritis and gill necrosis virus. J. Virol. 78, 9544–9551.

[11] Ronen, A., Perelberg, A., Abramowitz, J., Hutoran, M., Tinman, S., Bejerano, I., Steinitz, M. and Kotler, M. (2003) Efficient vaccine against the virus causing a lethal disease in cultured *Cyprinus carpio*. Vaccine 21, 4677–4684.

[12] Sano, T., Fukuda, H., Furukawa, M., Hosoya, H. and Moriya, Y. (1985) in: A Herpesvirus Isolated from Carp Papilloma in Japan (Ellis, A.E., Ed.), pp. 307–311, Academic Press, New York.

[13] Stephens, F.J., Raidal, S.R. and Jones, B. (2004) Haematopoietic necrosis in a goldfish (*Carassius auratus*) associated with an agent morphologically similar to herpesvirus. Aust. Vet. J. 82, 167–169.

[14] Hasegawa, S., Somamoto, T., Nakayasu, C., Nakanishi, T. and Okamoto, N. (1997) A cell line 9CFK0 from fin of isogeneic ginbuna crusian carp. Fish Pathol. 32, 127–128.

[15] Neukirch, M., Bottcher, K. and Bunnajirakul, S. (1999) Isolation of a virus from koi with altered gills. Bull. Eur. Assoc. Fish Pathol. 19, 221–224.

[16] Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D.J. (1997) Gapped BLAST and PSI-BLAST; a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.

[17] Meyer, H., Totmenin, A., Gavrilova, E. and Shchelkunov, S. (2005) Variola and camelpox virus-specific sequences are part of a single large open reading frame identified in two German cowpox virus strains. Virus Res. 108, 39–43.

[18] Smith, G.L., de Carlos, A. and Chan, Y.S. (1989) Vaccinia virus encodes a thymidylate kinase gene: sequence and transcriptional mapping. Nucleic Acids Res. 17, 7581–7590.

[19] Lee, N.S., Kobayashi, J. and Miyazaki, T. (1999) Gill filament necrosis in farmed Japanese eels, *Anguilla japonica* (Temminck & Schlegel), infected with *Herpesvirus anguillae*. J. Fish Dis. 22, 457–463.

[20] Davison, A.J. (1992) Channel catfish virus: a new type of herpesvirus. Virology 186, 9–14.

[21] Davison, A.J., Sauerbier, W., Dolan, A., Addison, C. and McKinnell, R.G. (1999) Genomic studies of the Lucke tumor herpesvirus (RaHV-1). J. Cancer Res. Clin. Oncol. 125, 232–238.

[22] Davison, A.J. (1998) The genome of salmonid herpesvirus 1. J. Virol. 72, 1974–1982.

[23] Bercovier, H., Fishman, Y., Nahary, R., Sinai, S., Zlotkin, A., Eyngor, M., Gilad, O., Eldar, A. and Hedrick, R.P. (2005) Cloning of the koi herpevirus (KHV) gene encoding thymidine kinase and its use for a highly sensitive PCR based diagnosis. BMC Microbiol. 5, 13.

[24] McGeoch, D.J., Rixon, F.J. and Davison, A.J. (2006) Topics in herpesvirus genomics and evolution. Virus Res. 117, 90–104.

[25] Rijsewijk, F., Pritz-Verschuren, S., Kerkhoff, S., Botter, A., Willemsen, M., van Nieuwstadt, T. and Haenen, O. (2005) Development of a polymerase chain reaction for the detection of Anguillid herpesvirus DNA in eels based on the herpesvirus DNA polymerase gene. J. Virol. Meth. 124, 87–94.