Molecular biology of fish viruses: a review

J Bernard, M Brémont

Vet Res (1995) 26, 341-351

Review article

Molecular biology of fish viruses: a review

J Bernard, M Brémont *

INRA, laboratoire de virologie et immunologie moléculaires, 78252 Jouy-en-Josas cedex, France

(Received 19 July 1994; accepted 29 June 1995)

Summary — The goal of this review is to present some of the recent molecular aspects in the fish virus studies. Although more than 50 different fish virus have been isolated and tissue-culture adapted, very few of them have been molecularly cloned and sequenced. Five virus families have been mostly studied: *Birnaviridae*, the prototype being the infectious pancreatic necrosis virus (IPNV), and the channel catfish virus (CCV) belonging to the *Herpesviridae* family. In the *Iridoviridae* family, the fish lymphocystis disease virus (FLDV) is the most studied. *Retroviridae* have been recently isolated and studied. The last family is the *Rhabdoviridae*, in which infectious hematopoietic necrosis virus (IHNV) and viral hemorrhagic septicemia virus (VHSV) have been extensively studied.

fish virus / molecular biology

Résumé — Biologie moléculaire des virus de poissons : une synthèse. *L'objet de cette revue est de présenter quelques aspects des connaissances récentes acquises au niveau moléculaire dans l'étude des virus de poissons. Bien que plus de 50 virus différents aient été isolés et adaptés à la culture cellulaire, très peu ont été étudiés par des approches moléculaires, tels que le clonage et le séquençage du génome. Cinq familles de virus ont plus spécialement été étudiées : les Birnaviridae dont le prototype chez les poissons est le virus de la nécrose pancréatique infectieuse (NPI), les Herpesviridae dont fait partie le virus du poisson-chat (CCV). Une autre famille très étudiée est celle des Iridoviridae, le virus de la maladie lymphocystique (FLDV) étant le plus connu, les Retroviridae récemment isolés chez les poissons et enfin les Rhabdoviridae dont les membres majeurs sont le virus de la nécrose hématopoiétique infectieuse (NHI) et le virus de la septicémie hémorragique virale (SHV).*

virus de poissons / biologie moléculaire

* Correspondence and reprints

INTRODUCTION

Interest in fish viruses has recently increased for a variety of reasons despite the fact that none of them infect warm-blooded animals. First, marine and fresh water aquaculture is expanding, and, consequently, epizootics are more frequent. New sanitary regulations for fish have been implanted, which include sampling for the detection of asymptomatic carriers. There is a need, therefore, for diagnostic molecular probes and, eventually, inexpensive vaccines made from recombinant proteins. Second, fish diverged from mammals about 400 million years ago and the viruses most probably co-evolved with their hosts. Thus, comparisons of fish virus genomes with those of viruses from other vertebrates is expected to reveal important information about genetic divergence during evolution. The identification of consensus sequences could also help to determine the primordial minimal sequence(s) necessary for functions such as enzyme activities, gene promoters and enhancers, or the assembly of different biological molecules such as nucleic acids and proteins.

More than 50 different fish viruses, all belonging to families already described for mammals, have been isolated in cell cultures. Their pathogenic, physical and chemical characteristics were reviewed by Wolf (1988) and Hetrick and Hedrick (1993). In this paper, we describe the recent developments concerning the molecular biology of fish viruses. In contrast with mammalian viruses, only a few have been studied from this point of view (table I). To our knowledge, the first sequences were published by Kiuchi and Roy (1984) and Roy *et al* (1984). Nevertheless, current sequence comparisons confirm the previous tentative classification of fish viruses, based on biological criteria, into subfamilies and genera different from the morphologically equivalent mammalian viruses. This likely indicates the evolutionary convergence of different encapsidation strategies. In contrast, short motifs are conserved which can be used as functional identifiers and could have been selected millenniums ago as being the most efficient.

BIRNAVIRIDAE

The *Birnaviridae* are the most extensively studied fish viruses. First of all because they are ubiquitous in aquatic organisms, since they have been isolated all over the world from both marine and freshwater fish of different species, and are responsible for severe losses in aquaculture. Secondly, their dsRNA genome is easily purified and is resistant to common RNases. Lastly, the

Table I. Fish viruses in which the genome has been at least partially cloned and/or sequenced.

Family	*Fish prototype virus*	*Genome*	*ORFs encoded*
Birnaviridae	IPNV	2 dsRNA	3, but 5 proteins
Herpesviridae	CCV	dsRNA	More than 79
Iridoviridae	FLDV	dsRNA	Unknown
Retroviridae	WDSV	RT activity	Unknown
Rhabdoviridae	IHNV	ssRNA	6

IPNV: infectious pancreatic necrosis virus; CCV: channel catfish virus; FLDV: fish lymphocystis disease virus; WDSV: walleye dermal sarcoma virus; IHNV: infectious hematopoietic necrosis virus.

birnaviruses have developed unusual coding strategies.

Infectious pancreatic necrosis virus (IPNV) is the prototype of the *Birnaviridae* (Dobos *et al*, 1979; Brown, 1986) which also includes the *Drosophila* X virus (DXV), the infectious bursal disease virus (IBDV) from poultry, other related viruses from aquatic organisms, such as the oyster virus (OV) and *Tellina tenuis* virus (TV), and a rotifer virus (RBV: Comps *et al*, 1991). These viruses differ from the bisegmented dsRNA viruses isolated from mammals, which are tentatively classified as Picobirnaviruses (Pereira *et al*, 1988), because of the size of their particle and their genome.

The single-shelled, naked, icosahedral, viral particle of birnaviruses contains 2 segments, A and B, of double-stranded RNA which are circularized by VP1, a viral protein (genome-linked protein, VPg) (DXV: Revet and Delain, 1982; IBDV: Müller and Nitschke, 1987; IPNV: Persson and MacDonald, 1982; Calvert *et al*, 1991). Coding assignments have been given to the 2 segments of DXV, IBDV and IPNV, and the segments of IPNV and IBDV have been sequenced. The organization of these genomes is similar (fig 1). Terminal repeats -AAGAG- are present at the 5' and 3' ends of the A segment and these are inverted on the B segment. These terminal sequences are identical for IPNV and IBDV and may play a role in replication or packaging.

The phenotypic divergence of these viruses has been described. For example, the relative electrophoretic mobilities of both the RNA and polypeptides may vary depending upon the geographic isolate (MacDonald and Gower, 1981). The eel virus European (EVE) strain is not pathogenic for salmonids (Sano *et al*, 1981), and the OV strain can multiply in the chinook salmon embryo (CHSE-214), but not in the fathead minnow (FHM) cell lines (MacDonald and Gower, 1981). Taking advantage of these divergences, the genetic analysis of the hybrids, obtained through coencapsidation of the A and B segments from different strains, has been used to demonstrate that segment B encodes VP1, while segment A encodes the structural proteins VP2 and VP3 (MacDonald and Dobos, 1981). One of the structural proteins encoded by segment A is the protein responsible for the host range in cultured cells (Darraghe and MacDonald, 1982). Moreover, when strain EVE is used as a donor for segment A, the resulting hybrid EVE/OV is not virulent for trout (Sano *et al*, 1992).

Segment A is 3 104 bp long for the virus strain N1 isolated in Sweden, from the Atlantic salmon *Salmo salar* (Havarstein *et al*, 1990) and 3 097 bp long for the virus strain Jasper isolated in Alberta, Canada, from the rainbow trout *Oncorhynchus mykiss* (Duncan and Dobos, 1986). The identity is 79.5% between the 2 IPNV isolates and 54% with IBDV. Two partially overlapping open reading frames (ORF) have been identified. A small one, located at the 5' end of the genome, encodes a 17 kDa (148 amino acids) protein (Havarstein *et al*, 1990), which

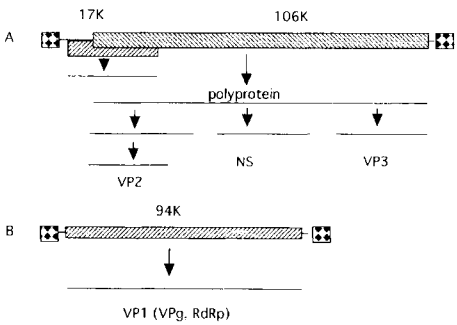

Fig 1. Organization of the genome of *Birnaviridae* and coding assignments (adapted from Dobos, 1992, International Symposium on Infectious Viruses in Fish, Seoul National University, Korea). A and B: RNA segments. Numbers indicate the deduced molecular weight of the protein in kDa. The successive cleavage products of the polyprotein are indicated by arrows.

can be immuno-precipitated from infected cells using an antiserum produced from rabbit immunization with the 17 kDa recombinant protein expressed in *Escherichia Coli* (Magyar and Dobos, 1992). The larger ORF (2 916 bp) encodes a 106 kDa polyprotein (Duncan and Dobos, 1986; Havarstein *et al*, 1990), which is co-translationally cleaved into the 3 major *Birnaviridae* gene products.

Hybrid arrested translation (HAT) shows that the protein order is 5'-VP2-NS-VP3-3' (Nagy *et al*, 1987). The autocatalytic proteolytic enzyme is identified as NS and the active site maps to its carboxyl-terminus (Duncan *et al*, 1987), between amino acids 693 and 720 (Manning *et al*, 1990). The precise cleavage sites on the precursor protein have not yet been identified.

It should be noted that the polyprotein has never been isolated from infected fish cells, since it is cleaved while synthesized. However, the fact that the *Birnaviridae* RNA-dependent RNA polymerase (RdRp) transcribes only unspliced RNAs (Bernard, 1980; Mertens *et al*, 1982; Spies *et al*, 1987) is in agreement with the presence of a single ORF on segment A.

Segment B from virus strains Sp (ubiquitous in Europe) and Jasper are 80.7% homologous (Duncan *et al*, 1991). A single ORF encodes the VP1 polypeptide of 844 (Sp) and 845 (Jasper) amino acids, with a homology of 88.6%. When compared with IBDV, as sequenced by Morgan *et al* (1988), the overall homology is only 41% between the 3 polypeptides.

Very little is known about the replication strategy of birnaviruses. VP1 is found in the viral particle both as a free form and bound to the RNA (VPg) and is thought to be the viral RNA dependent RNA polymerase. However, attempts to search for consensus sequences in the VP1 protein (Duncan *et al*, 1991) fail to find any known motifs such as the GDD motif, characteristic for the RNA polymerases. Comparison with the mammalian *Picobirnaviridae* would be useful, but no sequences have been published yet.

The motif G L P Y I G K T (IPNV) and GLPYVGRT (IBDV), reminiscent of the GXXXXGKS/T *ras*-type GTP binding proteins found on VP1, may relate to guanylyl transferase activity demonstrated for both IBDV (Spies and Müller, 1990) and IPNV (Dobos, 1993), which results in the formation of VP1-pG and VP1-pGpG. But, at least *in vitro*, the reaction is template dependent, is not reversible and is not inhibited by an excess of orthophosphate (Pi) or dithiotreitol (DTT). It is suggested, therefore, that the protein cannot act as a capping enzyme but is probably a primer during RNA synthesis (Dobos, 1993).

HERPESVIRIDAE

Several viruses isolated from fish have been classified as *Herpesviridae* on the basis of their biological, physical and biochemical properties (Wolf, 1988; Hedrick and Sano, 1989). The icosahedral capsid is made of 162 capsomeres that are embedded in a protein matrix and are surrounded by an envelope which includes virus-encoded glycoproteins. The capsid contains a single linear double-stranded DNA molecule, which is replicated in the nuclei of the infected cells. The high specificity of the host range suggests a closely related co-evolution.

Only 2 of the fish herpesviruses have been more extensively studied, these are the channel catfish herpesvirus (CCV) now known as ictalurid herpesvirus 1, isolated from *Ictalurus punctatus*, (Wolfe and Darlington, 1971) and *O masou* herpesvirus (OMV) now known as salmonid herpesvirus 2 (Roizmann, 1992), isolated from Pacific salmon (*O masou*) (Kimura *et al*, 1981 a,b).

The established physical map of the 53S CCV genome (Chousterman *et al*, 1979) shows that a unique region of about 95 kbp

is flanked at both ends by 18 kbp terminal repeats in the same orientation (direct terminal repeats, DTR) and there is no circular permutation. This structure (fig 2), which is also found for equine herpesvirus 2 and human herpesvirus 6 (HHV6), defines the herpesvirus group A (Roizmann *et al*, 1992).

The whole genome (134 226 bp) of one strain (Auburn 1) has been cloned and sequenced (Davison, 1992). The total nucleotide composition of 56.2% G + C is similar in the unique and repeat regions. Several short tandem direct reiterations are found, most of which are located in the DTRs. Computer analysis has permitted the identification of 79 protein-coding regions, among which ORFs 1 to 14 are located in the DTRs. Most genes are probably either transcribed from single exons or belong to families which presumably arose by gene duplication and have functional motifs in common, such as Zn-binding (ORFs 9, 11, 12) or protein kinase (ORFs 14, 15 and 16, ORFs 73 and 74). ORFs 62, 69 and 71 probably code after splicing for the terminase/packaging protein.

Despite the similarities with mammalian herpesviruses (virus particle structure, genome organization, fig 2) no homology was found between the predictable amino acid sequences of the proteins, except for short identifiers of functional motifs such as GNIGCG for thymidine kinase (ORF 5). A recent extensive comparison of the sequences of various DNA polymerases, the protein whose gene is supposed to be the most conserved among living organisms, showed that CCV is related to no other known organism (Ward, 1993).

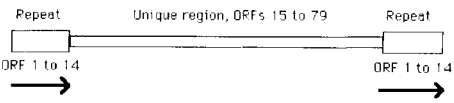

Fig 2. Organization of the genome of the channel catfish virus (CCV) (adapted from Roizmann *et al*, 1992).

In contrast, the deduced amino acid sequence from 2 EcoRI fragments of OMV (strain 007812) shows partial identity and similarity which permits their identification with ORFs 46 and 68 from CCV, despite the lack of homology on the nucleotide sequence (Bernard and Mercier, 1993). Thus CCV and OMV should clearly be classified together.

IRIDOVIRIDAE

Several fish viruses have been tentatively identified as iridoviruses based on biological and morphological criteria, but a single one, the fish lymphocystis disease virus (FLDV), has been extensively studied and has been classified, in the separate genus, lymphocystivirus of the *Iridoviridae*, (Francki *et al*, 1991). Different isolates have been classified into 2 strains, on the basis of the polypeptide patterns (Flügel *et al*, 1982), or restriction enzymes analysis and Southern blot hybridization (Darai *et al*, 1983). Strain 1 occurs in the fish species flounder *(Platichthys flesus)* and plaice *(Pleuronectes platessa)* and strain 2 occurs in dabs *(Limanda limanda)*.

The DNA extracted from the virus purified directly out of the papilloma-like lesions is highly methylated on the internal cytosine residues (Darai *et al*, 1983). The structure is linear but circularly permuted and terminally redundant (Darai *et al*, 1985), as has been described for other iridoviruses such as frog virus 3 (FV3), *chilo* iridescent virus (CIV) and tipula iridescent virus (TIV), so that the map is usually presented as a circle (fig 3).

The nucleotide sequence of the repetitive elements, thought to be associated with important regulatory functions during viral replication, is 94% homologous between strains 1 (EcoRI fragment M and left hand terminus of fragment B) and 2 (EcoRI fragments J and B), but different from that of other known *Iridoviridae* (Schnitzler *et al*,

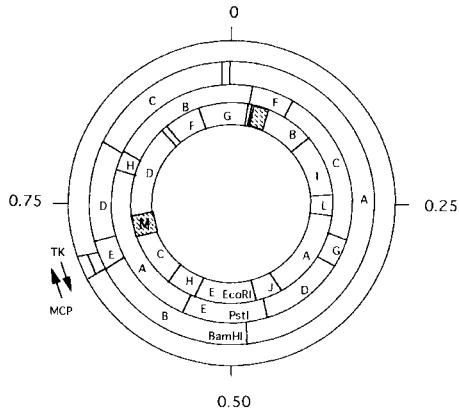

Fig 3. Organization of the genome of the fish lymphocystis disease virus (FLDV) (adapted from Schnitzler and Darai, 1993). TK: thymidine kinase. MCP: major capsid protein. Arrows: upper and lower strand. Hatched boxes: repeats. Numbers indicate the map units and letters the restriction fragments.

1987; Schnitzler and Darai, 1989). Each strand of the individual repetitive elements codes for a putative protein with classical glycosylation, promoter and termination signal sequences, but with no significant homology with other known proteins (Schnitzler and Darai, 1989). An attempt to use the repetitive elements to promote expression of chloramphenicol acetyl transferase (CAT) activity was successful in *E coli* in the correct, but not in the opposite orientation. However, the expression was not successful when mammalian-cultured cells were transfected (Schnitzler and Darai, 1989). The hypothesis that promoter activity is dependent upon the expression of other viral proteins has not been verified due to the poor efficiency of virus multiplication in cultured cells.

Thymidine kinase activity has been shown, by conversion of $Tk^{(-)}$ cells to $Tk^{(+)}$, to map between coordinates 0.675 and 0.691 (Scholz *et al*, 1988) and that region of the viral genome has been sequenced (Schnitzler *et al*, 1990, 1991). Only one motif, GNMSGYK, located at the amino acid position 283–289, is similar to the consensus GXXGXGK that is as common to all known Tks including the fish herpesviruses such as CCV.

In contrast, the major capsid gene, which maps between coordinates 0.669 and 0.718, could be cloned by PCR using primers which corresponded to the regions conserved for other iridoviruses (Schnitzler and Darai, 1993) Analysis of the deduced amino acid sequence shows a high degree of homology with TIV and CIV and identifies 3 hydrophobic motifs, one of which, EERR (amino acid 254–257), is conserved.

It should be noted that, as for typical *Birnaviridae*, no iridovirus is known for mammals, and thus comparison is impossible.

RETROVIRIDAE

C-type particles have often been described as observed through electron microscopy (Wolf, 1988), but it is only very recently that 2 viruses have been characterized in tumours.

Walleye dermal sarcoma virus (WDS) was isolated from a dermal tumour of *Stizostedion vitreum*. The RNA was extracted from the virus particles in gradient fractions which exhibited reverse transcriptase (RT) activity. These were molecularly cloned, using cDNA to probe for DNA in the tissues of tumour-bearing and control fish (Martineau *et al*, 1991). The 13.2 kbp DNA genome, found only in tumour-bearing fish, is unintegrated, linear, with long terminal repeats (LTR) and a central, single-stranded, gap structure at 5.6 kbp from the 3' end. Two major transcripts, 13 and 7.4 kb, are supposed to represent the full-length genomic message and the mRNA encoding the envelope protein respectively (Martineau *et al*, 1992).

A fish retrovirus has also been isolated by introgressive breeding of the platy fish *(Xiphophorus maculatus)* and the swordtail *(X helleri)*, leading to the development of embryos with whole-body melanomas. The embryos were used to establish a cell line, BsT, which after 40 passages in culture released in the supernatant viral particles with RT activity (Petry *et al*, 1992). The 70S RNA is packaged into virions which contain 6 proteins, 180, 120, 70, 65 and 28 kDa as analysed by PAGE. The 3 smaller proteins react with antiserum directed against the p27 feline leukemia virus (FeLV) protein, and thus may represent the *gag* precursor, its intermediate, and the major internal structural protein. Hybridization, using an FeLV LTR-gag DNA probe, with the DNA extracted from BsT cells and platy fish or swordtail, revealed homologous sequences. The *in vitro* RT activity is 40% inhibited by antiserum directed against FeLV RT. The use of the endogenous RT reaction products as probes detected 3 transcripts, 8.5, 4.2 and 1.5 kb in whole RNA from BsT cells and permitted the isolation of LTR structures from a genomic DNA library of *Xiphophorus*. This DNA library has no significant homology with other known, sequenced LTRs (Petry *et al*, 1992).

RHABDOVIRIDAE

Rhabdoviridae, identified as bullet-shaped, enveloped viruses with a single-stranded RNA genome of negative polarity, are frequently isolated from a wide range of host fish (see Wolf, 1988). Based on the electrophoretic pattern of the structural polypeptides, they are classified either as *Vesiculoviridae*, for example, spring viremia of carp virus (SVCV), or *Lyssaviridae,* for example, infectious haematopoietic necrosis virus (IHNV) and viral haemorrhagic septicaemia virus (VHSV). Of these, IHNV and VHSV have been the most extensively studied, due to their significant economic impact.

For SVCV, a 710 nucleotide insert has been sequenced. It codes for a single ORF of 223 amino acids, 28% homologous with the M (matrix) protein of vesicular stomatitis virus (VSV) San Juan strain Indiana (Kiuchi and Roy, 1984). In contrast, the first 20 nucleotides on the 3' end of the genome are almost completely homologous (Roy *et al*, 1984). To our knowledge, none of the other genes have been sequenced.

The genome of IHNV encodes 6 proteins in the order determined by R-loop mapping (3')N-M1-M2-G-Nv-L(5') (Kurath *et al*, 1985; Kurath and Leong, 1985). The *Nv* gene is remarkable in that it is not found in VSV and is only present as a remnant in the rabies virus (fig 4). In contrast, an *Nv* gene is found in other *Rhabdoviridae* such as bovine ephemeral fever (BEF) rhabdovirus (Walker *et al*, 1992) and Adelaide river (AR) rhabdovirus (Wang and Walker, 1993) or *Paramyxoviridae* (*HN* gene). A similar gene has also been identified on the genome of VHSV and sequenced (Basurco and Benmansour, personal communication). But while the BEF and AR virus *Nv* genes are most probably derived from the *G* gene by duplication (Wang and Walker, 1993), the VHS virus *Nv* gene sequence is related to no other gene.

In contrast to VSV or rabies, the leader sequence of the fish lyssaviruses is found even on clones obtained by reverse transcription of messenger RNA (Gilmore and Leong, 1988; Bernard *et al*, 1990). All the genes begin with the AACA sequence (fig

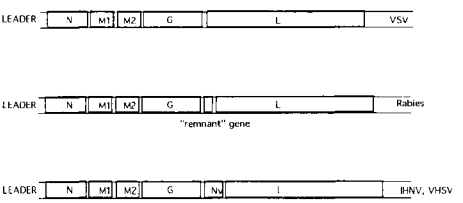

Fig 4. Organization of the genome of 3 *Rhabdoviridae*.

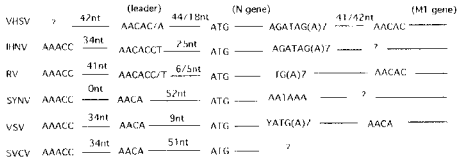

Fig 5. Start and stop signals for various *Rhabdoviridae*. VHSH: viral haemorrhagic septicaemia virus. IHNV: infectious haematopoietic necrosis virus. RV: rabies virus. SYNV: sonchus yellow net virus. VSV: vesicular stomatitis virus. SVCV: spring viremia of carp virus. Numbers indicate the number of nucleotides (nt).

5), which can thus be considered as the consensus transcription start signal for all *Rhabdoviridae*. The termination sequence AGATAG(A)7, found on fish lyssaviruses, is different from the consensus YATG(A)7 of vesiculoviruses and TG(A)7 of mammalian lyssaviruses (Benmansour *et al*, 1994). This heterogeneity may be related to the 'accidental' read-through frequently described on *Rhabdoviridae* transcripts, which have been interpreted as reflecting a poor efficiency of the termination signals.

The *N* protein gene has been sequenced for the round butte (RB) (Oregon, USA) isolate of IHNV (Gilmore and Leong, 1988), and the 07-71 (French, pathogenic) (Bernard *et al*, 1990) and Makah (Washington state, USA, asymptomatic) (Bernard *et al*, 1992) isolates of VHSV. Comparisons of the deduced amino acid sequences show 94.8% similarity and 91.1% identity between the *N* gene of the 2 VHSV strains, and 62.1 and 37.1% respectively, between VHSV and IHNV. The central portion of this protein, which is possibly attached to the genomic RNA, is the most highly conserved. In contrast, 20 nucleotides are deleted near the 3' end of the N protein of 07-71 as compared with Makah. This deletion is characteristic of all VHSV European pathogenic isolates studied so far and distinguishes them from the American asymptomatic isolates (Batts *et al*, 1993). Whether the deletion plays a role in its pathogenicity for the fish is yet unknown.

The G protein, which elicits the synthesis of neutralizing antibodies in injected fish (Engelking and Leong, 1989), has been sequenced for the RB IHNV (Koener *et al*, 1987) and the 07-71 (Thiry *et al*, 1991) and DK-3592B (Danish, pathogenic) (Lorenzen *et al*, 1993) VHSV isolates. The deduced polypeptide structure presents the classical signal peptide and transmembrane anchor segments. The 2 European VHSV isolates differ by only 13 amino acids (2.5%), all but one being located in the C terminal segment (258–462). Identity with IHNV is 38%, with strongly conserved domains such as 15 out of 16 cysteine residues in identical positions.

The *M1* and *M2* genes have been sequenced for only the 07-71 and Makah VHSV isolates (Benmansour *et al*, 1994). Amino acid identity between the 2 strains is respectively 89.7% for *M1*, 94.5% for *M2* (Benmansour *et al*, 1994) and 91% for *N* (Bernard *et al*, 1992). Both the *M1* and *M2* deduced polypeptides are highly basic. As for most other fish virus proteins, only the structural similarities are conserved when compared with other viruses from the same family.

CONCLUSION

As yet only a few fish virus genomes have been cloned and sequenced. Research interest focuses on viruses which have a severe impact on aquaculture and the aim of most laboratory research programs is to protect the fish. As expected, a number of fish pathologists are already using the published sequences to devise diagnostic probes and to screen fish populations to detect healthy carriers, or to trace the origins of local epidemics of disease.

The tentative models for evolution and taxonomy will need more data since, in most

cases, only 1 or 2 strains have been studied for each so-called family and it is not known whether they are representative.

The features shared by fish viruses and other viruses of the same putative family in the present classification, are the structure of the particle as determined by electron microscopy and the organization of their genome, such as the order of the different genes *(Rhabdoviridae)*, or the presence of LTRs (*Herpesviridae*, *Iridoviridae*, *Retroviridae*) or eventual single-stranded gaps (WDS). Thus, the classification into orders or 'super families' (ancient common ancestor) seems appropriate.

However these similarities are not reflected in the primary sequences of the genome and proteins. Significant homologies are only found for viruses such as *Birnaviridae* and *Iridoviridae*, which are not represented in mammals. Thus, insofar as sequence data are relevant for classification, new genera or even families will probably have to be created.

In contrast to this view, short functional identifiers on either nucleotide sequences (regulation of the transcription of mRNAs, by Tata boxes for DNA viruses), or amino acid sequences responsible for enzymatic activities (such as thymidine kinase or Zn binding) are conserved in both fish and mammalian viruses and their eucaryotic host. Such functional domains may have been subjected to strong selection as having been the most efficient since a primal ancestor. The RNA genomes, which have no cellular equivalent, contain more heterogeneous motifs such as transcription regulators.

ACKNOWLEDGMENTS

We acknowledge the helpful discussions with MK Estes (Houston) during the preparation of this manuscript.

REFERENCES

Batts WN, Arakawa CK, Bernard J, Winton JR (1993) Isolates of viral hemorrhagic septicemia virus from North America and Europe can be detected and distinguished by DNA probes. *Dis Aquat Org* **17** 67-71

Benmansour A, Paubert G, Bernard J, de Kinkelin P (1994) The polymerase-associated (M1) and the matrix protein (M2) from a virulent and an avirulent strains of Viral hemorrhagic septicemia virus (VHSV), a fish rhabdovirus. *Virology* 198, 602-612

Bernard J (1980) Drosophila X virus RNA polymerase: tentative model for *in vitro* replication of the double-stranded virion RNA. *J Virol* 33, 717-723

Bernard J, Lecocq-Xhonneux F, Rossius M, Thiry ME, de Kinkelin P (1990) Cloning and sequencing the messenger RNA of the N gene of viral haemorrhagic septicaemia virus. *J Gen Virol* 71, 1669-1674

Bernard J, Bremont M, Winton J (1992) Nucleocapsid gene sequence of a North American isolate of viral haemorrhagic septicaemia virus, a fish rhabdovirus. *J Gen Virol* 73, 1011-1014

Bernard J, Mercier A (1993) Sequence of two EcoRI fragments from Salmonis herpesvirus 2 and comparison with Ictalurid herpesvirus 1. *Arch Virol* 132, 437-442

Brown F (1986) The classification and nomenclature of viruses: summary of results of meetings of the International Comittee on Taxonomy of Viruses in Sendaï. September 1984. *Intervirology* 25, 140-143

Calvert JG, Nagy E, Soler M, Dobos P (1991) Characterization of the VPg-dsRNA linkage of infectious pancreatic necrosis virus. *J Gen Virol* 72, 2563-2567

Chousterman S, Lacasa M, Sheldrick P (1979) Physical map of the channel catfish virus genome: location of sites for restriction endonucleases EcoRI, HindIII, HpaI and XbaI. *J Virol* 31, 73-85

Comps M, Mari J, Poisson F, Bonami JR (1991) Biophysical and biochemical properties of the RBV, an unusual birnavirus pathogenic for rotifers. *J Gen Virol* 72, 1229-1236

Darai G, Anders K, Koch, HG *et al* (1983) Analysis of the genome of fish lymphocystis disease virus isolated directly from epidermal tumours of Pleuronectes. *Virology* 126, 466-479

Darai G, Delius H, Clarke J, Apfel H, Schnitzler P, Flügel RM (1985) Molecular cloning and physical mapping of the genome of fish Lymphocystis disease virus. *Virology* 146, 292-301

Darraghe A, MacDonald RD (1982) Host range restriction in infectious pancreatic necrosis virus maps to the large RNA segment and involves virus attachment to the cell surface. *Virology* 123, 264-272

Davison AJ (1992) Channel catfish virus: a new type of Herpesvirus. *Virology* 186, 9-14

Dobos P (1993) *In vitro* guanylylation of infectious pancreatic necrosis virus polypeptide VP1 *Virology* 193, 403-413

Dobos P, Hill B, Hallett R, Kells DTC, Becht H, Teninges D (1979) Biophysical and biochemical characterization of five animal viruses with bisegmented ds RNA genomes. *J Virol* 32, 593-605

Duncan R, Dobos P (1986) The nucleotide sequence of infectious pancreatic necrosis virus (IPNV) ds RNA segment A reveals one large ORF encoding a precursor polyprotein. *Nucleic Acids Res* 14, 5934

Duncan R, Nagy, E, Krell PJ, Dobos P (1987) Synthesis of the Infectious pancreatic necrosis virus polyprotein, detection of a virus-encoded protease, and fine structure mapping of genome segment A coding regions. *J Virol* 61, 3655-3664

Duncan R, Mason CL, Nagy E, Leong JA, Dobos P (1991) Sequence analysis of Infectious pancreatic necrosis virus genome segment B and its encoded VP1 protein: a putative RNA-dependent RNA polymerase lacking the Gly-Asp-Asp motif. *Virology* 181, 541-552

Engelking, H.M, Leong, J.A.C. (1989) The glycoprotein of Infectious hematopoietic necrosis virus elicits neutralizing antibody and protective responses. *Virus Res* 13, 213-230

Flügel RM, Darai G, Gelderblom H (1982) Viral proteins and adenosine triphosphate phosphohydrolase activity of fish lymphocystis disease virus. *Virology* 122, 48-55

Francki RIB, Fauquet CM, Knudson DL, Brown F (1991) Classification and nomenclature of viruses. *Fifth Report of the International Committee on Taxonomy of Viruses*, Springer-Verlag, Wien, 132-136

Gilmore RD, Leong, JAC (1988) The nucleocapsid gene of Infectious hematopoietic necrosis virus, a fish Rhabdovirus. *Virology* 167, 644-648

Havarstein LS, Kalland KH, Christie KE, Endresen C (1990) Sequence of the large double-stranded RNA segment of the N1 strain of infectious pancreatic necrosis virus: a comparison with other *Birnaviridae*. *J GenVirol* 71, 299-308

Hedrick RP, Sano T (1989) Herpesviruses of fish. *In: Viruses of Lower Vertebrates* (W Ahne, E Kurstak, eds), Springer-Verlag, Berlin, 161-171

Hetrick FM, Hedrick RP (1993) New viruses described in finfish from 1988-1992. *In: Annual Review of Fish Diseases* (M Faisal, FM Hetrick, eds) Pergamon Press, New York, 187-207

Kimura T, Yoshimizu M, Tanaka M, Sannohe H (1981a) Studies on a new virus (OMV) from *Oncorhynchus masou*. I. Characteristics and pathogenicity. *Fish Pathol* 15, 143-147

Kimura T, Yoshimizu M, Tanaka M (1981b) Studies on a new virus (OMV) from *Oncorhynchus masou* - II. Oncogenic nature. *Fish Pathol* 15, 149-153

Kiuchi A, Roy P (1984) Comparison of the primary sequence of spring viremia of carp virus M protein with that of vesicular stomatitis virus. *Virology* 134, 238-243

Koener JF, Passavent CW, Kurath G, Leong JA (1987) Nucleotide sequence of a cDNA clone carrying the glycoprotein gene of infectious hematopoietic necrosis virus, a fish rhabdovirus. *J Virol* 61, 1342-1349

Kurath G, Leong JAC (1985) Characterization of infectious hematopoietic necrosis virus mRNA species reveals a non-virion Rhabdovirus protein. *J Virol* 53, 462-468

Kurath G, Ahern KG, Pearson GD, Leong JAC (1985) Molecular cloning of the six mRNA species of infectious hematopoietic necrosis virus, a fish Rhabdovirus, and gene order determination by R-loop mapping. *J Virol* 53, 469-476

Lorenzen N, Olesen NJ, Jorgensen PEV, Etzerodt M, Holtet TL, Thogersen HC (1993) Molecular cloning and expression in *Escherichia coli* of the glycoprotein gene of VHS virus, and immunization of rainbow trout with the recombinant protein. *J Gen Virol* 74, 623-630

MacDonald RD, Dobos P (1981) Identification of the proteins encoded by each genome segment of Infectious pancreatic necrosis virus. *Virology* 114, 414-422

MacDonald RD, Gower DA (1981) Genomic and phenotypic divergence among three serotypes of aquatic birnaviruses (infectious pancreatic necrosis virus) *Virology* 114, 187-195

Magyar G, Dobos P (1994) Evidence for the detection of the infectious pancreatic necrosis virus polyprotein and 17 kDa polypeptide in infected cells and of the NS protease in purified virus. *Virology* 204, 580-589

Manning DS, Mason CL, Leong JC (1990) Cell-free translational analysis of the processing of infectious pancreatic necrosis virus polyprotein. *Virology* 179, 9-15

Martineau D, Renhaw RR, Williams JR, Casey JW, Bowser PR (1991) A large unintegrated retrovirus DNA species present in a dermal tumor of walleye *Stizostedion vitreum*. *Dis Aquat Org* 10, 153-158

Martineau D, Bowser PR, Renhaw RR, Casey JW (1992) Molecular characterization of a unique retrovirus associated with a fish tumor. *J Virol* 66, 596-599

Mertens PPC, Jamieson PB, Dobos P (1982) *In vitro* RNA synthesis by Infectious pancreatic necrosis virus-associated RNA polymerase. *J Gen Virol* 59, 47-56

Morgan MM, MacReadie IG, Harley, VR, Hudson PJ, Azad AA (1988) Sequence of the small double-stranded RNA genomic segment of infectious bursal disease virus and its deduced 90 kDa product. *Virology* 163, 240-242

Müller H, Nitschke R (1987) The two segments of the Infectious bursal disease virus genome are circularized by a 90 000 Da protein. *Virology* 159, 174-177

Nagy E, Duncan R, Krell P, Dobos P (1987) Mapping of the large RNA genome segment of infectious pan-

creatic necrosis virus by hybrid arrested translation. *Virology* 158, 211-217

Pereira HG, Flewett TH, Candeias JAN, Barth OPM (1988) A virus with a bisegmented double-stranded RNA genome in rat (*Oryzomys nigripes*) intestines. *J Gen Virol* 69, 2749-2754

Persson RH, MacDonald RD (1982) Evidence that infectious pancreatic necrosis virus has a genome-linked protein. *J Virol* 44, 437-443

Petry H, Petry K, Schmidt M, Hunsmann G, Anders F, Lüke W (1992) Isolation and characterization of a retrovirus from the fish genus *Xiphophorus*. *Virology* 188, 785-792

Revet B, Delain E (1982) The Drosophila X virus contains a 1 μM double standard RNA circularized by a 67 kDa terminal protein: high resolution denaturing mapping of its genome. *Virology* 123, 29-44

Roizmann B, Desrosiers RC, Fleckenstein B, Lopez C, Minson AC, Studdert MJ (1992) The family *Herpesviridae*: an update. *Arch Virol* 123, 425-449

Roy P, Gupta KC, Kiuchi A (1984) Characterization of spring viremia of carp virus mRNA species and the 3' sequence of the viral RNA. *Virus Res* 1, 189-202

Sano T, Okamoto N, Nishimura T (1981) A new viral epizootic of *Anguilla japonica* Temmick and Schlegel. *J Fish Dis* 4, 127-139

Sano M, Okamoto N, Fukuda H, Saneyoshi M, Sano T (1992) Virulence of infectious pancreatic necrosis virus is associated with the larger RNA segment (RNA segment A). *J Fish Dis* 15, 283-293

Schnitzler P, Darai G (1989) Characterisation of the repetitive DNA elements in the genome of fish lymphocystis disease viruses. *Virology* 172, 32-41

Schnitzler P, Darai G (1993) Identification of the gene encoding the major capsid protein of fish lymphocystis disease virus. *J GenVirol* 74, 2143-2150

Schnitzler P, Delius H, Scholz J, Touray M, Orth E, Darai G (1987) Identification and nucleotide sequence analysis of the repetitive DNA element in the genome of fish lymphocystis disease virus. *Virology* 161, 570-578

Schnitzler P, Rözen-Wolff A, Darai G (1990) Molecular biology of fish lymphocyctis disease virus. *In: Molecular Biology of Iridoviruses* (G Darai, ed), Kluwer Academic Publishers, Boston, USA, 203-234

Schnitzler P, Handermann M, Szepe O, Darai G (1991) The primary structure of the thymidine kinase of fish lymphocystis disease virus. *Virology* 182, 835-840

Scholz J, Rösen-Wolff A, Touray M, Schnitzler P, Darai G (1988) Identification, mapping and cloning of the thymidine kinase gene of fish lymphocystis disease virus. *Virus Res* 9, 63-72

Spies U, Müller H (1990) Demonstration of enzyme activities required for cap structure formation in infectious bursal disease virus, a member of the birnavirus group. *J Gen Virol* 71, 977-981

Spies U, Müller H, Becht H (1987) Properties of RNA polymerase activity associated with infectious bursal disease virus and characterization of its reaction products. *Virus Res* 8, 127-140

Thiry M, Lecocq-Xhonneux F, Dheur I, Renard A, de Kinkelin P (1991) Sequence of e cDNA clone carrying the glycoprotein gene and part of the matrix protein *m2* gene of viral haemorrhagic septicaemia virus, a fish rhabdovirus *Biochim Biophys Acta* 1090, 345-347

Walker PJ, Byrne KE, Riding GA, Cowley JA, Wang Y, MacWilliam S (1992) The genome of bovine ephemeral fever Rhabdovirus contains two related glycoprotein genes. *Virology* 191, 49-61

Wang Y, Walker, PJ (1993) Adelaide river Rhabdovirus expresses consecutive glycoprotein genes as polycistronic mRNAs: new evidence of gene duplication as an evolutionary process. *Virology* 195, 719-731

Ward C W (1993) Progress towards a higher taxonomy of viruses. *Res Virol* 144, 419-453

Wolf K (1988) *In: Fish Viruses and Fish Viral Diseases.* Comstock Publishing Associates, Cornell University Press, Ithaca, USA

Wolfe D, Darlington RW (1971) Channel catfish virus: a new herpesvirus of ictalurid fish. *J Virol* 8, 525-533