

# Retroviruses of wild and cultured fish

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## Abstract

Retroviruses comprise a large group of enveloped RNA viruses which have been found in a wide range of vertebrate species including fish. To date a number of fish retrovirus genomes have been partially or completely sequenced. Phylogenetic analysis and genome organization indicate a high diversity of fish retroviruses as well as some unique structural features that have not been found in any other retroviruses. Piscine retroviruses comprise both exogenous and endogenous viruses; most of them are associated with proliferative diseases. Because several of these proliferative diseases have a seasonal trend, they provide an excellent model for studying tumor development and regression. The aim of this work was to review the best-described fish retroviruses.

**Key words:** fish, retroviruses, tumor

## Introduction

All genetic elements that contain a gene encoding reverse transcriptase (RT) are referred to as retroelements or retroids (Hull 2001, Basta et al. 2009). According to the classification proposed by Hull (2001) retroelements comprise two major groups, *Retrovirales* which consist viral elements and *Retrales* containing non-viral elements. *Retrovirales* are split into three suborders, *Orthoretrovirineae* which contain retroviruses (with RNA genome), *Pararetrovirineae* (viruses with DNA genome) and the *Retrotransposineae*, consisting of retrotransposons (these elements can form virus-like particles although without the possibility to move from cell to cell). The order *Retrales* is subdivided into retroposons (non-LTR elements, e.g. LINES-long interspersed repetitive elements) and retrons (distinct DNA sequences which synthesise an un-

usual satellite DNA known as msDNA- multi-copy single-stranded DNA) (Hull 2001, Hansen and Heslop-Harrison 2004, Lampson et al. 2005).

Retroviruses are the best known viral retroelements. In accordance with ICTV (International Committee on Taxonomy of Viruses) retroviruses belong to the family *Retroviridae*, although a formal taxonomic proposal was made to create a new classification for all reverse transcribing elements (Hull 2010). The family *Retroviridae* is not assigned to an order and consists of two subfamilies, *Orthoretrovirinae* and *Spumaretrovirinae*. To date, six genera have been defined in the subfamily *Orthoretrovirinae* (alpha-, beta-, gamma-, delta, epsilon-, lentivirus) and one genus in the subfamily *Spumaretrovirinae* (spumavirus). *Epsilonretrovirus* is the most recent genus added to the family *Retroviridae* and comprises three fish retroviruses: walleye dermal sarcoma virus (WDSV) and

Table 1. Retroviruses occurring in fish.

Virus name	Abbreviation	Original host species	References
Walleye dermal sarcoma virus	WDSV	<i>Sander vitreus</i>	Walker 1969
Walleye epidermal hyperplasia virus type 1	WEHV 1	<i>Sander vitreus</i>	Walker 1969
Walleye epidermal hyperplasia virus type 2	WEHV 2	<i>Sander vitreus</i>	Walker 1969
Perch epidermal hyperplasia virus type 1	PEHV 1	<i>Perca flavescens</i>	Quackenbush et al. 2001
Perch epidermal hyperplasia virus type 2	PEHV 2	<i>Perca flavescens</i>	Quackenbush et al. 2001
Salmon swimbladder sarcoma virus	SSSV	<i>Salmo salar</i>	Duncan 1978
Salmon leukemia virus	SLV	<i>Oncorhynchus tshawytscha</i>	Kent et al. 1990
Snakehead retrovirus	SnRV	<i>Ophicephalus striatus</i>	Frerichs et al. 1991
Zebrafish endogenous retrovirus	ZFERV	<i>Danio rerio</i>	Shen and Steiner 2002

walleye epidermal hyperplasia viruses type 1 and type 2 (WEHV-1, WEHV-2). Other fish retroviruses have not yet been assigned to a specific genus (Basta et al. 2009). The summary data for fish retroviruses are shown in Table 1.

Retroviruses are enveloped RNA viruses infecting all groups of vertebrates. By using reverse transcriptase (RT) they transcribe their genomic RNA into double stranded DNA and insert it into the infected host cell genome. The viral particles are composed of core (capsid) containing RNA genome, viral enzymes and an outer envelope. The envelope is made of lipid bilayer (derived from the plasma membrane of the host cell) in which viral glycoproteins are embedded. Their genome is diploid, monomers are held together by hydrogen bonds. Retroviruses are the only viruses, which contain two identical RNA molecules incorporated into one viral particle. Viral RNA is linear, single-stranded, in positive-sense orientation. The 3' end of each monomer is polyadenylated. The 5' terminus has a methylated nucleotide cap and tRNA, which is base-paired to a region, termed the primer-binding site (PBS). This tRNA is used as a primer for replication. Based on genome organisation retroviruses are divided into two categories: simple and complex. All retroviruses contain three major coding domains: *gag*, *pro-pol* and *env*, flanked by regulatory regions known as long terminal repeats (LTRs). Complex retroviruses encode additionally proteins, which play a regulatory role. The *gag* (group-specific antigen) gene codifies for structural polyprotein containing matrix protein (MA), capsid protein (CA) and nucleocapsid protein (NC). The *pro* (protease) gene encodes an enzyme, which is responsible for cleavage of *gag* and *gag/pol* precursors during the maturation pro-

cess. The *pol* (polymerase) gene codifies for a poly-protein comprised of a reverse transcriptase with ribonuclease activity (RT/RNaseH) and an integrase (INT) which is essential in the process of viral integration into the host genome. The *env* (envelope) gene encodes the envelope glycoprotein, which is spliced in the maturation process into the outer surface (SU) membrane protein (major virus antigen), and the transmembrane (TM) protein (Vogt 1997a, b, Galetto and Negroni 2009, Gallo and Reitz 2010).

The retroviral infection begins when the viral surface glycoprotein recognizes and binds to the receptor of a susceptible host cell. After the fusion of viral and host cell membranes, the viral particle enters the cell and loses its envelope and capsid. In the cytoplasm viral reverse transcriptase converts the viral RNA into intermediate DNA.

Translocation into the nucleus and integration into the genomic DNA (mediated by integrase) is the next step in the retroviral life cycle. Virus genome integrated into the host cell DNA is referred to as a provirus. Transcription and translation of the mRNA into viral proteins proceed using the host cell machinery. Newly assembled virion buds from the host cell membrane and matures; a new round of infection may occur (Galetto and Negroni 2009, Gallo and Reitz 2010).

There are two major viral strategies of transmission: horizontal and vertical. Most retroviruses infect somatic cells, are transmitted horizontally and they are termed exogenous viruses. Occasionally, when the infection occurs in the germ line, the retroviral genes become a part of the host genome and the virus is transmitted vertically. These viruses are referred to as endogenous retroviruses (ERVs). The

genome of most of the endogenous retroviruses is defective (they are unable to replicate independently) (Rasmussen 1997, Gifford and Tristem 2003).

### Retroviruses associated with skin tumors in walleyes

Walleye dermal sarcoma virus (WDSV) is etiologically associated with skin tumors in walleye (*Sander vitreus*), a freshwater perciform fish native to most regions of North America. Walleye dermal sarcoma (WDS) was first reported in 1969 on fish collected from Oneida Lake in New York State. WDS lesions occur as benign, cutaneous mesenchymal neoplasms, randomly distributed on the fish. They range in size from 0.2 to 1 cm in diameter (Walker 1969). Unusually large lesions (from 2-3.5 cm in diameter) with malignant character were occasionally observed on wild adult walleyes (Bowser et al. 2002). The most characteristic feature is that the disease has a seasonal trend, appearing in late fall and naturally regressing during the spring spawning period. There is a strong correlation between regression of WDS and water temperature. When the water temperature increases the tumors regress, as observed in nature and experimentally infected fish (Bowser et al. 1990, Getchell et al. 2000, Rovnak and Quackenbush 2010). Transmission of WDS virus in nature occurs by direct contact of walleyes during the spring spawning season or through contact with water containing the virus (Bowser et al. 1999, Rovnak and Quackenbush 2010).

The disease affects annually up to 27% of adult walleyes in Oneida Lake and 10% of walleyes in Canada (Quackenbush et al. 2001).

In addition to the three major coding domains (*gag*, *pro-pol* and *env*), the WDSV genome contains three open reading frames: *orf A* and *orf B*, located between *env* and 3' LTR and an open reading frame designated *orf C*, situated between 5' LTR and the *gag* gene. These additional genes encode the retroviral accessory proteins (Holzschu et al. 1995, Holzschu et al. 1997, Rovnak and Quackenbush 2010). Orf A codifies for a protein that is distantly related to cellular D-type cyclins (19% sequence identity with D1 human cyclin), and therefore it was named retroviral cyclin (rv-cyclin) (Lapierre et al. 1998a, Holzschu et al. 2003). The localisation of rv-cyclin in the nucleus and its physical association with transcription factors suggest a role in transcription regulation and cell proliferation, which may result in tumor development. Lapierre et al. (1998a) suggested that the WDSV rv-cyclins may promote cell cycle progression by activating cyclin-dependent kinases (Cdks). Cdks initiate the phosphorylation cascades that culminate in cell division.

The study shows that WDSV Orf A induces cell cycle progression in yeast (*Saccharomyces cerevisiae*) deficient in G1/S cyclins, supporting this conjecture (Lapierre et al. 1998a). In contrast to these studies Paul et al. (2011) demonstrated that rv-cyclin is not independently sufficient to induce tissue proliferation in transgenic, rv-cyclin-expressing zebrafish. Tumor induction has not been observed even after exposure to chemical mutagens (Paul et al. 2011). Corresponding with these data, Zhan et al. (2010) suggested that rv-cyclin protects the liver from carcinogen damage and delays tumor development in transgenic zebrafish. Rv-cyclin may also block NF- $\kappa$ B and interferon transcription in order to avoid cellular and humoral immune responses (Rovnak and Quackenbush 2010).

Orf B protein localizes in the cytoplasm and plasma membrane (Rovnak et al. 2007). WDSV Orf B directly interacts with the receptor for activate C kinase (RACK1) which leads to the activation of the protein kinase C signaling pathway. PKC activation may contribute to cell survival and proliferation. RV-cyclin and Orf B probably cooperate to cause efficient tumor formation (Daniels et al. 2008, Rovnak and Quackenbush 2010).

WDSV Orf C protein has been localized in mitochondria and this localisation has been correlated with the induction of apoptosis, which contributes to tumor regression (Nudson et al. 2003, Rovnak and Quackenbush 2010).

WDSV is experimentally transmissible to sauger (*Sander canadensis*) and yellow perch (*Perca flavescens*) (Holzschu et al. 1998, Bowser et al. 2001).

Walleye epidermal hyperplasia viruses type 1 and type 2 (WEHV-1 and WEHV-2), like walleye dermal sarcoma virus, belong to the genus Epsilonvirus and is a causative agent of walleye epidermal hyperplasia. Walleye epidermal hyperplasia is a hyperproliferative skin disease characterized by broad, flat plaques of thickened epidermis, with distinct boundaries. The WEH lesions may occur on any part of the body and fins, but are most commonly situated on the caudal fin. They range in size from 2 to 50 mm in diameter (Walker 1969, Rovnak and Quackenbush 2010). WEH has been observed annually on approximately 10% of adult walleyes in Oneida Lake, N.Y., and on up to 20% of walleyes in Canadian lakes (Lapierre et al. 1998b). Direct contact during the spring spawning period is a route of natural transmission of WEHV-1 and WEHV-2 (Plumb and Hanson 2011).

WEHV-1 and WEHV-2 are large viruses, closely related to one another, with 77% amino acid identity within the *pol* region and 95% identity within the RT region. WEHV-1 and WEHV-2 also share 83% and 81% amino acid identity with WDSV, respectively,

within the region of polymerase (Lapierre et al. 1998b).

Like the WDSV, WEH exhibit a seasonal cycle, lesions appear in the autumn, start to regress in the spring and are absent during the summer months. The genome organisation and transcriptional profiles of WEHV-1 and WEHV-2 are similar to those of the walleye dermal sarcoma virus. Like the WDSV, these walleye retroviruses contain the three open reading frames *orfA*, *orfB* and *orfC* in addition to *gag*, *pro-pol* and *env*. Sequence analysis of WEHV1 and WEHV2 *orfAs* shows that they also encode D-type cyclin homologs. The *orfA* proteins are distantly related to *orfB* proteins, suggesting that they could have arisen by gene duplication and their origin can be traced to a cellular gene (Lapierre et al. 1998a,b, Lapierre et al. 1999).

WDSV, WEHV1 and WEHV2 use similar strategies of viral replication and induction of cell proliferation. They are the first example of retroviruses that encode cyclin homologs and may represent a new class of oncogenic retroviruses (Lapierre et al. 1998a).

### **Perch epidermal hyperplasia virus type 1 and type 2 (PEHV-1, PEHV-2)**

Perch epidermal hyperplasia virus type 1 and type 2 are retroviruses associated with hyperplastic lesions found on yellow perch (*Perca flavescens*). These lesions are similar to walleye epidermal hyperplasias and occur as thickened plaques on the fish body. PEHV has been partially sequenced, and based on differences between LTRs, was divided into two types: PEHV-1 and PEHV-2. Genome organisation is presumably similar to those of WEHV-1, WEHV-2 and WDSV. PEHV-1 and PEHV-2 are tentative members of the *Epsilonretrovirus* genus (Quackenbush et al. 2001, Fauquet and Mayo 2005).

### **Salmon swimbladder sarcoma virus (SSSV)**

The first outbreak of neoplastic disease involving the swim bladder of Atlantic salmon (*Salmo salar*) was observed in 1976, at a commercial fish farm in Scotland (Duncan 1978, McKnight 1978). The affected fish were in poor physical condition, had swollen abdomens and presented multinodular masses on the external and internal surfaces of the swim bladder. Histological examination showed that tumors arose at the junction of the inner smooth muscle and the areolar tissue zone of the swim bladder. The tumors were classified as fibrosarcomas (Duncan 1978) or leiomyosarcomas (McKnight 1978). Examination of

affected tissue by electron microscopy revealed retrovirus-like particles (Duncan 1978).

The second outbreak of swim bladder sarcoma was noticed in 1996 in juvenile salmon collected from the Pleasant River in Maine and maintained at the North Attleboro National Fish Hatchery in Massachusetts (Paul et al. 2006). Diseased animals exhibited physical symptoms similar to those of the previous report on salmon swim-bladder sarcoma. Histologically, the multinodular masses localizing on or replacing the swim bladder were composed of well-differentiated fibroblastic cells arranged in interlacing bundles (Paul et al. 2006). By the spring of 1998, cumulative mortality reached 35% of the population. Samples provided from the affected fish were used to obtain a complete nucleotide sequence of the virus (Paul et al. 2006). They found that SSSV is a simple retrovirus, 10.9 kb in length, containing three open reading frames (*gag*, *pro-pol*, *env*) flanked by LTR sequences at each end of the genome. Phylogenetic analysis based on *pol* conserved regions suggests that SSSV is most closely related to ZFERV (zebrafish endogenous retrovirus), revealing 40% identity within the central region of the *pol* gene. Salmon swim bladder sarcomas exhibit a very high proviral copy number (greater than 30 copies per cell), suggesting a high level of SSSV expression, which leads to multiple infections of tumor cells. Experimental transmission using cell free virus homogenates to naive Atlantic salmon has been successful, although tumors were not observed. Thus, it is possible that other factors (e.g. host, environmental conditions) may play a role in the etiology of salmon swim bladder sarcoma (Paul et al. 2006).

### **Salmon leukemia virus (SLV)**

Plasmacytoid leukemia (PL) was first observed in 1988, in chinook salmon (*Oncorhynchus tshawytscha*) commercially reared in seawater netpens in British Columbia, Canada (Kent et al. 1990). Affected fish were anaemic and lethargic, with swollen abdomens; some of them displayed prominent bilateral exophthalmia. Further histological examination revealed massive proliferation and infiltration of plasmacytoid cells in the kidney interstitium, spleen, pancreas, liver and heart (Kent et al. 1990).

Eaton and Kent (1992) suggested that the plasmacytoid leukemia has a retroviral etiology. They showed that tissues collected from fish with PL exhibit RT (reverse transcriptase) activity associated with retrovirus-like particles, observed by electron microscopy. The virus was designated salmon leukemia virus (SLV) (Eaton and Kent 1992). Frequent co-infec-

tions of PL-affected fish with microsporidium *Enterocytozoon salmonis* and *Renibacterium salmoninarum* suggested that these agents may act as co-factors in the development of PL (Eaton et al. 1994). However, transmission investigations indicated that the disease can be transmitted in the absence of these pathogens (Kent and Dawe 1990, 1993). Also, further studies of wild-caught chinook salmon with PL symptoms did not reveal *E. salmonis* in any of the examined fish (Eaton et al. 1994).

The study of Eaton et al. (1994) has shown the presence of SLV in wild-caught populations of chinook salmon collected from the strait of Georgia, Canada.

Plasmacytoid leukemia was experimentally transmitted to healthy chinook salmon by using intraperitoneal injection of tissue homogenates. Transmission with cell-free filtrates was equivocal, since none of the fish from this experimental group exhibited histological signs typical of PL, although one fish exhibited nests of cells in the liver. Experimental interspecies transmission of PL was achieved in sockeye salmon (*O. nerka*) and Atlantic salmon (*S. salar*) (Kent and Dawe 1990, Newbound and Kent 1991).

The origin of salmon leukemia virus is still unknown. Some studies suggest that the virus may derive from previously unexpressed endogenous retroviral DNA sequences (Eaton et al. 1994). Preliminary evidence that the PL is transmitted vertically and a similar model of origin in some of the murine leukemia viruses support this hypothesis (Eaton et al. 1994).

### Snakehead retrovirus (SnRV)

The snakehead retrovirus was first reported as a spontaneously productive infection of fish cell line SSN-1 derived from striped snakehead fish (*Ophicephalus striatus*). Examination by electron microscopy revealed C-type virus particles. Cell culture supernatants demonstrated high levels of RT activity and induced a cytopathic effect in the BF-2 cell line derived from bluegill fry (*Lepomis macrochirus*). Potential pathogenicity of SnRV is under investigation. All fish from which these cell lines were derived appeared clinically healthy (Frerichs et al. 1991).

Experimental infection of juvenile snakehead fish with SnRV showed no lesions in any of the infected fish (Frerichs et al. 1993).

The SnRV genome obtained from cell line (SSN-1) has been cloned and sequenced by Hart et al. (1996). Genomic organisation and transcriptional profile indicate that the SnRV virus belongs to complex

retroviruses, although it is unique in comparison to any known complex fish retroviruses. Except for typical *gag*, *pro-pol* and *env* open reading frames flanked by LTR's, SnRV has 3'ORF open reading frame located between *env* and 3'LTR and two small open reading frames designated ORF1 and ORF2. The function of these accessory proteins in the virus life cycle remains unknown. The amino acid sequence data of the ORFs proteins showed no significant homology to any viral and non-viral proteins. The structure of the *gag*, *pro-pol* and *env* coding regions also displayed some unusual domains of unknown function. Phylogenetic analysis, based on the RT amino acid alignment, presents a distant relationship between SnRV and the mammalian C-type retroviruses (Hart et al. 1996). Southern-blot analysis suggests that SnRV is an exogenous virus (Hart et al. 1996, Rovnak and Quackenbush 2010).

### Zebrafish endogenous retrovirus (ZFERV)

Zebrafish endogenous retrovirus was originally isolated from the thymus of zebrafish (*Danio rerio*) (Shen and Steiner 2004). The provirus of ZFERV was detected in the sperm of different fish at the same genetic locus, indicating that it is an endogenous virus. While most of endogenous viruses genes are not transcript due to mutations and deletions, ZFERV genes seem to be intact and transcriptionally active. The highest level of viral transcript expression was found in the larval and adult zebrafish thymus. The genome of ZFERV is 11.2 kb in length, and, like all retroviruses, contains three principal genetic domains (*gag*, *pro-pol*, *env*), flanked by LTRs. *Gag* and *pro-pol* genes are in the same open reading frame. Phylogenetic analysis has shown that ZFERV is closest to murine leukemia virus (MLV)-related retroviruses and to walleye fish retroviruses, although genome structure is more similar to MLV-related retroviruses (Shen and Steiner 2004).

### Conclusions

Genetic analysis has shown that fish retroviruses are a highly diverse group compared to other retroviruses (Basta et al. 2009). Some of the fish retroviruses exhibit unique features, such as those associated with skin tumors in walleyes which encode cyclin homologs. Walleye retroviruses encoding these accessory proteins seem to be a useful model for studying tumorigenesis. Most of the discovered fish retroviruses are oncogenic. The diseases provoked by these retroviruses occur and regress on a seasonal basis, so

they may serve as a unique model system to investigate cancer development and regression. Because the number of recognized fish retroviruses is increasing, the diseases caused by these pathogens need further investigation. Pathogenicity and viral transmission studies, as well as development of reliable diagnostic methods, seem to be the most important for the fish industry.

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