Infectious Pancreatic Necrosis Virus

Subjects: Virology

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Infectious pancreatic necrosis (IPN) is a disease of great concern in aquaculture, mainly among salmonid farmers, since losses in salmonid fish—mostly very young rainbow trout (Salmo gairdnery) fry and Atlantic salmon (Salmo salar) post-smolt—frequently reach 80–90% of stocks. The virus causing the typical signs of the IPN disease in salmonids, named infectious pancreatic necrosis virus (IPNV), has also been isolated from other fish species either suffering related diseases (then named IPNV-like virus) or asymptomatic; the general term aquabirnavirus is used to encompass all these viruses. Aquabirnaviruses are non-enveloped, icosahedral bisegmented dsRNA viruses, whose genome codifies five viral proteins, three of which are structural, and one of them is an RNA-dependent RNA polymerase.

IPNV virulence Aquabirnavirus

1. The Structure and General Characteristics

The infectious pancreatic necrosis virus (IPNV) is an unenveloped icosahedral virus with an average size of around 65 nm, as stated by the International Committee for Taxonomy of Viruses (ICTV) for the general characteristics of the family *Birnaviridae* [1], or 60 nm as reviewed by Munro and Midtlyng [2]. However, a diameter ranging from 57 to 74 nm was reported in an early review by Dobos and Roberts [3], which is more in accordance with the results of a recent study by Lago et al. [4], where a range of sizes between 55 and even 90 nm were visualized in different fractions of a purified IPNV West Buxton type virus (and other type strains), the most frequently observed size being around 70 nm. The virion, of a molecular weight of 55×10^6 Da, shows an approximate protein/RNA content rate of 91/9, a buoyant density in CsCl of 1.33g/ml, and a sedimentation coefficient of 435 S. But one of the main features of this virus is its high stability to physicochemical conditions: pH (stable at pH values as low as 3), salinity (from 0‰ to 40‰), and temperature (resistant to up to 60 °C for 30 min, and able to replicate from 4 to 27.5 °C) as reviewed by Wolf [5] and more recently by Munro and Midtlyng [2].

Regarding the classification of IPNV strains or—in general terms of aquatic birnaviruses—two approaches are applied (see revisions [2][6]). The first classification, based on serological typing, was definitively outlined by Hill and Way [7]. They classified the aquabirnaviruses into two serogroups (A and B) and nine serotypes within serogroup A (**Table 1**). For the second approach, in spite of the high number of reports providing a diverse classification [2], there is a consensus to consider as definitive the typing into six genogroups proposed by Blake et al. [8] and later extended by Nishizawa et al. [9] with a seventh (**Table 1**). There is a correspondence between serotype, genotype, and type strain: the American strains WB (West Buxton) and Ja (Jasper), from USA and Canada, respectively, would constitute genotype 1, corresponding to serotypes A1 and A9, respectively; the Danish type strains Sp

(Spajarup) and Ab (Abildt) constitute serotypes A2 and A3, and genotypes 5 and 2, respectively; Hetch (He), originally from Germany, is serotype A4 and genotype 6; genotype 3 is constituted by isolates clustering with type strains Te (Tellina, from UK; serotype A5) and C1 (Canada 1; serotype A6), and type strains C2 and C3 (from Canada, corresponding to serotypes A7 and A8) constitute genotype 4; finally, the seventh genotype corresponds to Japanese marine birnavirus (MaBV).

Table 1. Serotyping and genotyping of aquatic birnavirus.

Stp [1]		Gtp [2]	Type Strain	Geogr Origin [3]
A	A1	1	WB	USA
	A2	5	Sp	Denmark
	A3	2	Ab	Denmark
	A4	6	He	Germany
	A5	3	Te	UK
	A6	3	C1	Canada
	A7	4	C2	Canada
	A8	4	C3	Canada
	A9	1	Ja	Canada
B	B1	–	TV-1	UK
		7	MaBV [4]	Japan

1—Serogroup/Serotype; 2—Genotype; 3—Geographic origin; 4—Marine birnavirus.

2. The Viral Genome

The genome is constituted by two segments of dsRNA named A and B. Although the virion was traditionally thought to contain a single set of both segments, it is now known to be polyploid, able to package 1, 1.5, and even 2 genome equivalents [4]. Segment A, with a size between 2962 and 3097 bp [2], contains two open reading frames (ORF; **Figure 1**). The ORF PP, the largest one, encodes a polyprotein of around 106 kDa, which comprises most of the viral proteins: NH_2-pVP2-VP4-VP3-COOH. Due to the internal proteolytic activity of the VP4 region, this polyprotein suffers a co-translational modification consisting in a protease cleavage, between an alanine and a serine, in two positions: between amino acids 734 and 735, to release the minor capsid protein VP3, and between amino acids 508 and 509, to produce the non-structural viral proteins VP4 and pVP2; this is an immature precursor of the major structural protein VP2 which, during morphogenesis, suffers proteolytic cleavage creating the mature structural form and three additional small peptides which remain associated to the virion [10]. The second ORF of this segment, ORF VP5, codifying the non-structural VP5 protein, is in fact the first one since it overlaps ORF PP and its start codon precedes the ORF PP start codon by a few nucleotides. Finally, segment B, of around 2400 bp, contains a single ORF encoding the minor structural protein VP1.

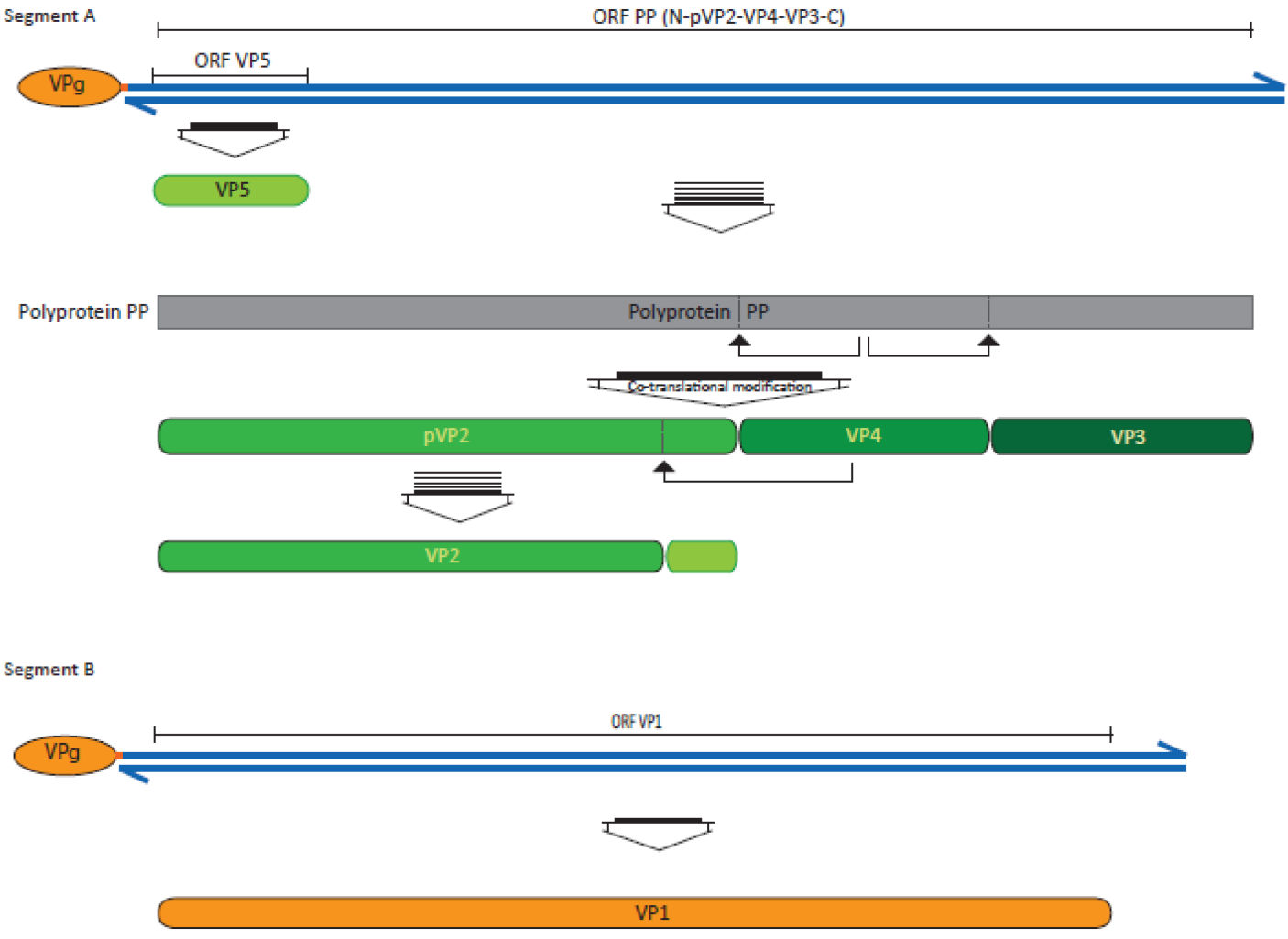

Figure 1. Genome organization and expression.

The 5′- and 3′-ends of the viral genome have untranslated terminal repeats (5′- and 3′-UTR) with an important implication in several steps of the viral replication, as well as in virulence. To this regard, as first reported by Dobos [11] and Magyar et al. [12], the 5′-ends of both segments are the sites for the covalent cell attachment of the structural VP1 protein, which, in this attached form, is named VPg to differentiate it from the free VP1 form. Mutations in this 5′-UTR were known to affect IPNV infectivity [13], and Boot et al. [14] suggested that, since birnaviruses lack a 5′-cap and—because of the short length of the 5′-UTR—also lack an internal ribosome entry site (IRES) to use the cell-encoded initiation factors, the VPg linked at the 5′-end would be involved in initiation of translation. However, it has also been reported that 5′-UTR lengths shorter than the normal 300 nt are not necessarily an impediment to constitute an IRES structure, and Rivas-Aravena et al. [15] have demonstrated that the 5′-UTR forms a functional structure, efficiently acting as an IRES, which commands translation.

At the other end of the strands, the 3´-UTR is known to be involved in second strand RNA synthesis. The infectious bursal disease virus (IBDV; a member of the related genus *Avibirnavirus* frequently used as reference for IPNV structure and replication) is known to have a couple of cytosines at this end allowing the VPg to act as a protein-primer thanks to its linked guanines; but to function, the 3′-UTR must maintain a specific stem-loop structure [15]. In the case of the mRNA, since its 3′-end lacks a poly(A) tail to simulate the cellular mRNA and thus to defend against exonuclear activity, and enhance its translation, its function is probably substituted by the 3′-UTR stem loop structure [14].

3. The Viral Proteins and Their Function

The viral genome encodes five viral proteins named from VP1 to VP5. Three of them (VP1, VP2, and VP3) are structural, and to date two (VP4 and VP5) are believed to be non-structural proteins. An ORF encoding a putative sixth 25 kDa viral protein has been found by Shivappa et al. [16] overlapping part of the ORF PP, between the pVP2 and VP4 sequences. They found that sequence exclusively in the Sp strain, and later a similar sequence was also found in a few Chilean strains. Due to the presence of positively-charged domains, it was speculated to interact with the inner plasmatic membrane leaflet [17].

VP1, the viral protein encoded by the genome segment B (with an approximate size of 94 kDa), is the viral RNA-dependent RNA-polymerase (RdRp) needed for genome replication and transcription [18]. As previously described, this structural protein is present in two forms: VP1, free inside the capsid, and VPg, linked to the 5′-ends of both genomes [2], probably only to the plus strands [12].

The same VP1 polypeptide may act as an RdRp and a protein-primer for the initiation of RNA synthesis. For that purpose, VP1 suffers a self-guanylylation, without the need of a template RNA to produce VP1pGpG [19][20]. The cytosines at the 3′-end termini of the genome will allow that molecule to act as the protein-primer for the synthesis of a second RNA strand. Consequently, VP1 remains linked to the 5′-end of the new synthetized strand, thus becoming VPg.

VP2, a structural protein of about 54kDa [6], is, in number of units, the main component of the capsid. Its structure and organization have been recently well defined by Coulibaly et al. [21][22] (**Figure 2**). It is constituted of three domains (**Figure 2**A): a central one—called S—which, in subviral particles (SVPs) constitutes the shell (**Figure 2**B); the base (B), which is located in the inner side of the particle, and the spike or projection (P) to the outside of the capside. The spikes—organized in VP2 trimers around a 3-fold axis (**Figure 2**C)—contain the main viral antigenic sites, the cell specificity epitope, and some of the virulence determinants, as described in more detail below.

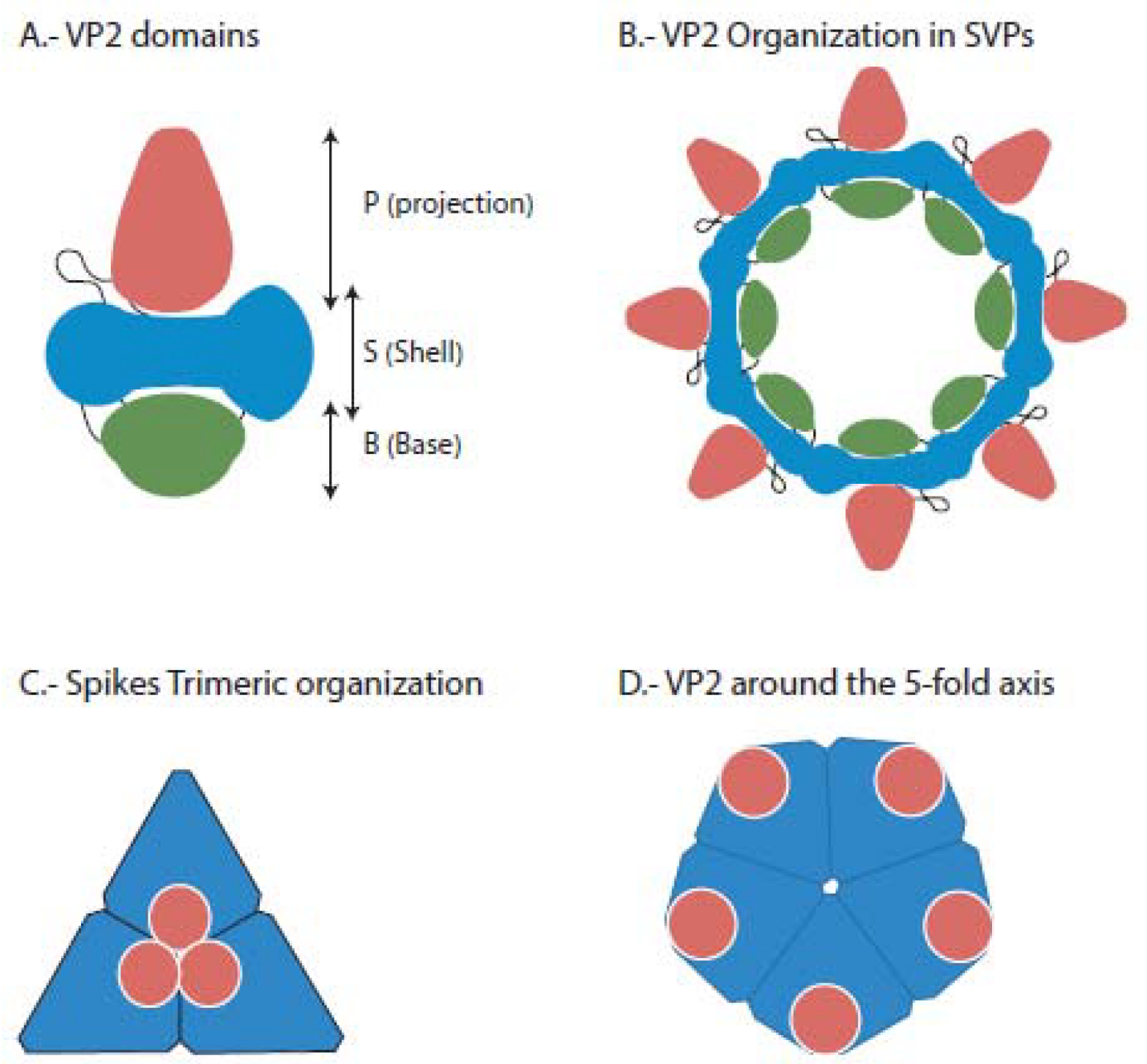

Figure 2. General structure and organization of the VP2 protein in infectious pancreatic necrosis virus (IPNV). (**A**) VP2 is structurally constituted by three domains: a central one (blue), which in subviral particles (SVPs) constitutes the shell (see panel (**B**)) and is named before that (S); the base (B; green), which is located in the inner side of the

particle, and the spike or projection (P, red), on the other side. (**B**) Structural organization of VP2 in SVPs. (**C**) Trimeric organization of the spikes. (**D**) Organization of the VP2 units around the 5-fold axis [23].

Before that study, VP2 was already known to carry the cell attachment sites and to be responsible for most of the antigenicity of the virus. As early as in the late 1980s, Caswel-Reno et al. [23] demonstrated the presence of neutralization epitopes in VP2, and Azad et al. [24] located the same type of epitopes in an internal region of VP2—between amino acids 206 and 350—which was recognized by the virus-neutralizing monoclonal antibody (MAb) 17/82 (**Figure 3**). That section of VP2 was soon recognized by Håvarstein et al. [25] to be a hypervariable region in the VP2 sequence, almost coinciding with the widely known "central variable domain" described by Heppell et al. [26]. Other studies using MAbs confirmed VP2 to be the main protein responsible for IPNV antigenicity, and in one of them Dobos [27] found a serotype-specific epitope, also located in that central region of the molecule.

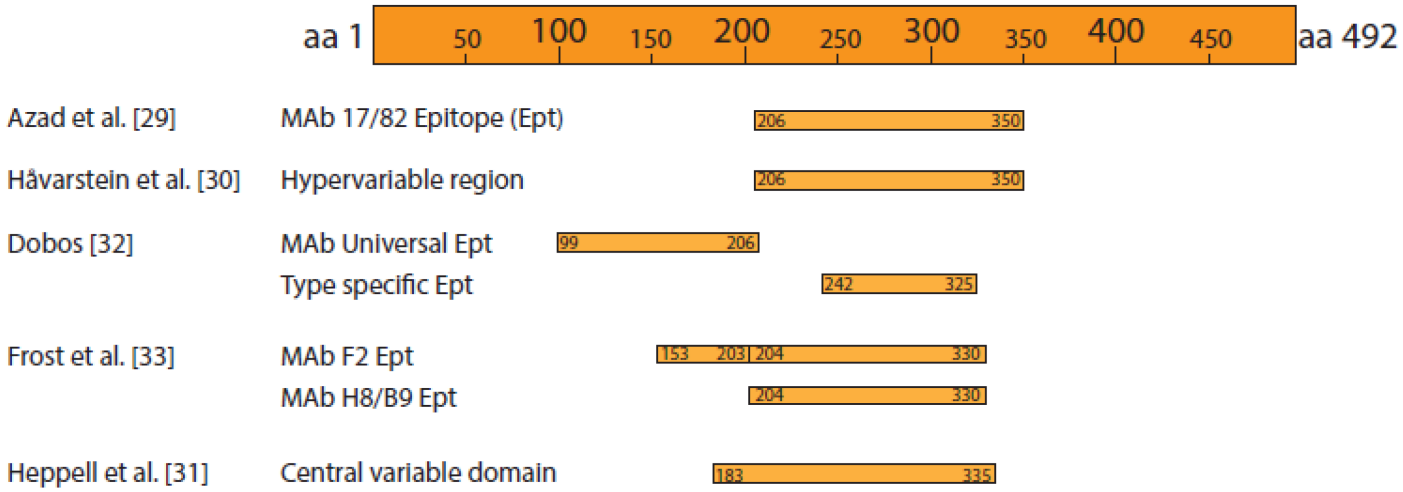

Figure 3. VP2 epitopes and variable region map.

Even prior to discovering its antigenicity [28], VP2 was known to be the viral protein in charge of cell-attachment and was therefore considered responsible for cell and host specificity [29]. The attachment site is located in the P domain of VP2 (**Figure 2**A) at the top of the spike [22]; however, certain amino acids located at the groove of the spike, close to domain S, are also important for binding to the cell, at least in IBDV [30]. Therefore, Coulibaly et al. [22] suggested that those two units could correspond to two different receptors, the first, at the top of the spike, would be for cell recognition and attachment, and the one in the spike's groove would oversee cell internalization.

The viral proteins involved in cell attachment are frequently glycosylated, since that type of maturation confers special properties to the viral protein, which are important in the process of adsorption to cells and, therefore, in cell tropism and virulence. There are two types of glycosylation; the most common, the N-linked glycosylation, corresponds to a post-translational modification taking place inside the endoplasmic reticulum (ER); the second one, the O-glycosylation, reported to occur freely in the cytoplasm, has been demonstrated to be important for IPNV integrity and infectivity [25][31][32][33].

VP3 is a relatively small molecule with several functions. As reviewed in a recent study where the authors reported that IBDV-VP3 upregulates the RNA synthesis activity by the viral RdRp [34], VP3 is a multifunctional protein which also acts as a scaffolding protein during morphogenesis and provides protection against the anti-viral cell response; additionally, it has been reported to induce apoptosis in fish [35]. Vp3, first thought to be a trimeric [36] and later dimeric [37] protein, is the second component of the capsid in terms of copy numbers, behind VP2, the major one. VP3 is known to be an internal viral protein in IPNV since Dobos [27] discovered that not only the genome but also this protein were absent in empty capsids. Nevertheless, part of the protein must be exposed to the outer side of the capsid since some reaction with neutralizing MAbs has been demonstrated [38]. VP3 interacts with VP1, and also captures the viral genome, constituting a ribonucleoprotein (RNP) structure which protects the viral RNA against the host defenses [39][40]. For this VP3–dsRNA binding, the N-terminal of the protein is crucial, although both the C- and N-end domains are important for the binding [39]. Although Bahar et al. [41] reported that this association had no effect on VP1 activity, soon after Ferrero et al. [34] demonstrated that VP3 did actually have an upregulating effect on the VP1-mediated RNA replication. Additionally, the VP1–VP3 complex promotes the assembly of pVP2 units, constructing a precursor capsid which, after the maturation of pVP2 by proteolysis to VP2 (and three small peptides), will constitute the mature capsid [39][40].

VP4 is the viral protein whose autoproteolytic activity cleaves the polyprotein PP during its own translation. This is VP4's most known function, but this protein is also known to trans-activate the synthesis of VP1 [42], although the mechanism is still unknown; and, more recently [43] it has been discovered that VP4 also strongly inhibits interferon induction, and such antagonistic activity is not linked to its proteolytic activity.

VP5 is a non-structural protein codified by the small ORF at the 5′-end of segment A. There is a certain discrepancy about the size of this protein, undoubtedly due to the high variability observed. The size of this protein in IPNV was originally reported to be of 17 kDa [44], similar to that of IBDV [45], although a size of 15 kDa was also reported, since two start codons [46] can appear in the 5′-end sequence of that segment (**Figure 4**), one in nucleotide (nt) position 68, and a second in nt 112, depending on the strain. However, in 2001 Webber et al. [13] demonstrated that, in IPNV, VP5 used the second start codon position, encoding a 15 kDa (399 nt/133 aa) protein [16][47], although a truncated 12 kDa VP5 has been frequently detected in some strains [16][48]. In addition, a shorter truncated form of only 28 aa, due to the presence of a premature stop-codon, has also been reported [47].

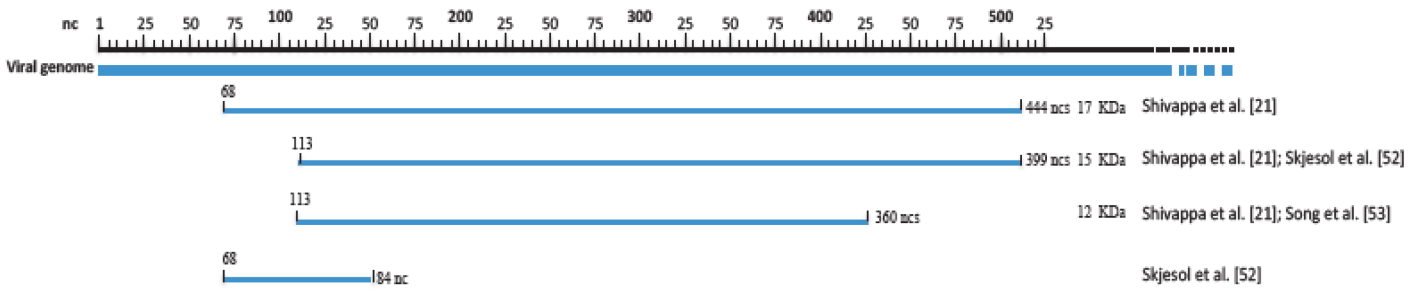

Figure 4. VP5 protein open reading frame (ORF).

The function of this protein has not been definitively defined yet. Although it is considered non-essential for virus replication both in vitro [13] and in vivo [49], it has been revealed to regulate protein expression in the early steps of viral replication, and even to enhance cell viability by preventing membrane rupture and DNA cleavage, as reported by Hong et al. [50], which in fact enhances progeny production. Such antiapoptotic activity is widely accepted for IBDV–VP5 [51], but there is much controversy surrounding the antiapoptotic function of this protein in IPNV. To this regard, in spite of the homology of IPNV–VP5 with the Bcl-2 antiapoptotic proteins, previously reported [50] and demonstrated by Ortega et al. [52], these authors concluded that VP5 had no influence on apoptosis. This supported previous results by Santi et al. [49]; however, in their paper they recognized that the mutations observed in putatively important locations in the domain of the strains under study could be an explanation for the lack of activity. Therefore, IPNV–VP5 is still accepted as an antiapoptotic protein [53][54], which also has a strong inhibition of the interferon (IFN) signaling [43][55]. Finally, in IBDV, in addition to this antiapoptotic activity at early stages of infection, VP5 has been demonstrated to activate cell apoptosis in late stages, possibly constituting a mechanism for progeny release [56], something that could be similar in IPNV.

4. Replication Cycle

Understanding the structure of the virus, as well as the function of its components is a prerequisite to recognizing the strategies it uses to modulate virulence.

Therefore, the researchers will now look into the way the virus infects and replicates in a susceptible cell (see reviews [2][57]). A complete replication cycle takes between 16 and 20 h. In the first step, adsorption, VP2 acts as the attachment viral receptor to specifically recognize the surface cell receptor. In CHSE-214 cells, up to 6000 of those receptors have been calculated to be available for IPNV fixation [58], although just a quarter of them would provide a specific attachment. VP2 has two domains involved in adsorption [22], one at the top of the spike, used for specific fixation to the cell receptor, and a second at the groove, close to the bottom of the spike, involved in the internalization step. Adsorption takes around 20 min, internalization is produced via receptor-mediated endocytosis, and in just 2 h p.i. new synthesized RNA (a transcription intermediate) is detected. Since dsRNA is susceptible to being identified as exogenous by the cell defenses, it was thought to be protected like in the case of reovirus infection, which carries out transcription and replication inside a viral core. To this regard, RdRp activity has been associated to IPNV virions without proteolytic treatment [57]. However, an RNP made up by a VP1–VP3–RI (RNA intermediate) complex is also known to be involved in RNA synthesis [39][40]. In fact, both types of RNPs have been demonstrated to play a role in RNA synthesis [59], and to protect the new synthesized viral RNA against cell defenses. The progeny genome synthesis starts between 4 and 6 h p.i. and protein synthesis is also observed during that time; the maximum genome synthesis is reached 8–10 h p.i and declines at 14–16 h p.i. [57]. The VP3 protein in the VP1–VP3–dsRNA RNP complex acts as a scaffolding protein [39][40], capturing pVP2 to construct an immature non-infectious 68 nm particle. This particle, which appears after 8 h p.i., at the beginning of the morphogenesis phase, suffers a maturation process to be transformed (12 h p.i.) into the 60 nm infective virion [60]; this maturation consists of the proteolytic cleavage of pVP2 into VP2 by the activity of VP4, although the

participation of cell proteases cannot be dismissed [27]. Finally, if the internalization of the viral progeny is as reported for IBDV [61][62], VP5 would accumulate in the cell membrane, triggering its lysis and the viral release.

5. IPNV and Persistence

A widely known fact is that the reservoirs of an episode become lifelong asymptomatic carriers, in which the virus can be detected—in certain tissues—mainly after stress episodes. Initially, it was accepted that the virus used a high proportion of defective interfering (DI) particles to create a balance between infective viral particles and host defenses. DI particles have the capacity to attach and penetrate into a susceptible cell, but not to replicate because they lack part of their genome. Therefore, their replication depends on co-infection with the wild type infectious virus, and their defective parental genome generates identical defective progeny genome copies. In cell culture, the presence of a high proportion of DI particles in an inoculum has been demonstrated to interfere with IPNV replication and progeny production, creating persistently infected monolayers [2]. Marjara et al. [63] also recently demonstrated—in persistently infected cells—the upregulation of genes involved in transcription repression, which suggests that IPNV persistence could be due to the reduction of viral replication as a result of a reduced transcription capacity. Other authors [54][64] have added that the persistence status could be due to the virus and host reaching a balance in such a manner that the virus reduces its level of replication so as not to harm the host and thus not triggering the host defenses.

References

1. Delmas, D.; Attoui, H.; Ghosh, S.; Malik, Y.S.; Mundt, E.; Vakharia, V.N. ICTV Report Consortium, ICTV Virus Taxonomy Profile: Birnaviridae. J. Gen. Virol. 2019, 100, 5–6.
2. Munro, E.S.; Midtlyng, P.J. Infectious pancreatic necrosis virus and associated aquatic birnavirus. In Fish Diseases and Disorders Vol. 3: Viral, Bacterial and Fungal Infections, 2nd ed.; Woo, P.T.K., Bruno, D.W., Eds.; CAB International: Cambridge, MA, USA, 2011; pp. 1–65.
3. Dobos, P.; Roberts, T.E. The molecular biology of infectious pancreatic necrosis virus: A review. Can. J. Microbiol. 1983, 29, 377–384.
4. Lago, L.; Rodríguez, J.F.; Bandín, I.; Dopazo, C.P. Aquabirnavirus polyploidy: A new strategy to modulate virulence? J. Gen. Virol. 2016, 97, 1168–1177.
5. Wolf, K. Fish Viruses and Fish Viral Diseases; Comstock Pbl Ass., Cornwell Univ. Press: New York, NY, USA, 1988; p. 576.
6. Reno, P.W. Infectious pancreatic necrosis virus and associated aquatic birnavirus. In Fish Diseases and Disorders, Vol. 3: Viral, Bacterial and Fungal Infections; Woo, P.T.K., Bruno, D.W., Eds.; CAB International: New York, NY, USA, 1999; pp. 1–55.

7. Hill, B.J.; Way, K. Serological classification of infectious pancreatic necrosis (IPN) virus and other aquatic birnaviruses. Ann. Rev. Fish Dis. 1995, 5, 55–77.
8. Blake, S.L.; Ma, J.-Y.; Caporale, D.A.; Jairath, S.; Nicholson, B.L. Phylogenetic relationships of aquatic birnaviruses based on deduced amino acid sequences of genome segment A cDNA. Dis. Aquat. Org. 2001, 45, 89–102.
9. Nishizawa, T.; Kinoshita, S.; Yoshimizu, M. An approach for genogrouping of Japanese isolates of aquabirnaviruses in a new genogroup, VII, based on the VP2/NS junction region. J. Gen. Virol. 2005, 86, 1973–1978.
10. Galloux, M.; Chevalier, C.; Henrry, C.; Huet, J.C.; Costa, B.D.; Delmas, B. Peptides resulting from the pVP2 C-terminal processing are present in infectious pancreatic necrosis virus particles. J. Gen. Virol. 2004, 85, 2231–2236.
11. Dobos, P. Protein-primed RNA synthesis in vitro by the virion-associated RNA polymerase of infectious pancreatic necrosis virus. Virology 1995, 208, 19–25.
12. Magyar, G.; Chung, H.K.; Dobos, P. Conversion of VP1 to VPg in cells infected with infectious pancreatic necrosis virus. Virology 1988, 245, 142–150.
13. Weber, S.; Fichner, D.; Mettenleiter, T.C.; Mundt, E. Expression of VP5 of infectious pancreatic necrosis virus strain VR299 is initiated at the second in-frame start codon. J. Gen. Virol. 2001, 82, 805–812.
14. Boot, H.; Pritz-Verschuren, B.E.P. Modifications of the 3′UTR stem-loop of infectious bursal disease virus are allowed without influencing replication or virulence. Nucl. Acids Res. 2004, 32, 211–222.
15. Rivas-Aravena, A.; Muñoz, P.; Jorquera, P.; Diaz, A.; Reinoso, C.; González-Catrilelbún, S.; Sandino, A.M. Study of RNA-A Initiation Translation of The Infectious Pancreatic Necrosis Virus. Virus Res. 2017, 240, 121–129.
16. Shivappa, R.; Song, H.; Yao, K.; Aas-Eng, A.; Evensen, Ø.; Vakharia, V.N. Molecular characterization of Sp serotype strains of infectious pancreatic necrosis virus exhibiting differences in virulence. Dis. Aquat. Org. 2004, 61, 23–32.
17. Gondenberg, N.M.; Steinberg, B.E. Surface charge: A key determinant of protein localization and function. Cancer Res. 2010, 70, 1277–1280.
18. Villanueva, R.A.; Guacucano, M.; Pizarro, J.; Sandino, A.M. Inhibition of virion-associated IPNV polymerase, VP1, by radiolabeled nucleotide analogs. Virus Res. 2005, 112, 132–135.
19. Dobos, P. In vitro guanylation of infectious pancreatic necrosis virus polypeptide VP1. Virology 1993, 193, 403–413.

20. Xu, H.-T.; Si, W.D.; Dobos, P. Mapping the site of guanylylation on VP1, the protein primer for infectious pancreatic necrosis virus RNA synthesis. Virology 2004, 322, 199–210.
21. Coulibaly, F.; Chevalier, C.; Gutsche, I.; Pous, J.; Navaza, J.; Bressanelli, S.; Delmas, B.; Rey, F.A. The birnavirus crystal structure reveals structural relationships among icosahedral viruses. Cell 2005, 120, 761–772.
22. Coulibaly, F.; Chevalier, C.; Delmas, B.; Rey, F.A. Crystal Structure of an Aquabirnavirus Particle: Insights into Antigenic Diversity and Virulence Determinism. J. Virol. 2010, 84, 1792–1799.
23. Caswell-Reno, P.; Reno, P.W.; Nicholson, B. Monoclonal antibodies to infectious pancreatic necrosis virus: Analysis of viral epitopes and comparison of different isolates. J. Gen. Virol. 1986, 67, 2193–2205.
24. Azad, A.A.; Jagadish, M.N.; Brown, M.A.; Hudson, P.J. Deletion mapping and expression in Escherichia coli of the large genomic segment of birnavirus. Virology 1987, 161, 145–152.
25. Håvarstein, L.S.; Kalland, K.H.; Christie, K.E.; Endresen, C. Sequence of the large double-stranded RNA segment of the NI strain of infectious pancreatic necrosis virus: A comparison with other Birnaviridae. J. Gen. Virol. 1990, 71, 299–308.
26. Heppell, J.L.; Tarrab, E.; Lecomte, J.; Berthiaume, L.; Arella, M. Strain variability and localization of important epitopes on the major structural protein (VP2) of infectious pancreatic necrosis virus. Virology 1995, 214, 40–49.
27. Dobos, P. The molecular biology of infectious pancreatic necrosis virus (IPNV). Ann. Rev. Fish. Dis. 1995, 5, 25–54.
28. Frost, P.; Håvarstein, L.S.; Lygren, B.; Stahl, S.; Endresen, C.; Christie, K.E. Mapping of neutralization epitopes on infectious pancreatic necrosis virus. J. Gen. Virol. 1995, 76, 1165–1172.
29. Darragh, E.A.; MacDonald, R.D. A host range restriction in infectious pancreatic necrosis virus maps to the large RNA segment and involves virus attachment to the cell surface. Virology 1982, 123, 264–272.
30. Delgui, L.; Ona, A.; Gutierrez, S.; Luque, D.; Navarro, A.; Caston, J.R.; Rodríguez, J.F. The capsid protein of infectious bursal disease virus contains functional alpha 4 beta 1 integrin ligand motif. Virology 2009, 386, 360–372.
31. Hjalmarsson, A.; Everitt, E. Identification of IPNV- specified components released from productively infected RTG-2 cell following massive cytopathic effect. Arch. Virol. 1999, 144, 1487–1501.
32. Espinoza, J.C.; Hjalmarsson, A.; Everitt, E.; Kuznar, J. Temporal and subcellular localization of infectious pancreatic necrosis virus structural proteins. Arch. Virol. 2000, 145, 739–748.

33. Fridholm, H.; Everitt, E. Virion glycosylation governs integrity and infectivity of infectious pancreatic necrosis virus. J. Fish Dis. 2011, 34, 663–675.
34. Ferrero, D.; Garriga, D.; Navarro, A.; Rodríguez, J.F.; Verdaguer, N. Infectious Bursal Disease Virus VP3 Upregulates VP1-Mediated RNA- Dependent RNA Replication. J. Virol. 2015, 89, 11165–11168.
35. Chiu, C.-L.; Wu, J.-L.; Her, G.-M.; Chou, Y.-L.; Hong, J.-R. Aquatic birnavirus capsid protein, VP3, induces apoptosis via the Bad-mediated mitochondria pathway in fish and mouse cells. Apoptosis 2010, 15, 653–668.
36. Boot, H.J.; ter Huurne, A.A.H.M.; Hoekman, A.J.W.; Pol, J.M.; Gielkens, A.L.J.; Peeters, B.P.H. Exchange of the C-Terminal Part of VP3 from Very Virulent Infectious Bursal Disease Virus Results in an Attenuated Virus with a Unique Antigenic Structure. J. Virol. 2002, 76, 10346–10355.
37. Casañas, A.; Navarro, A.; Ferrer-Orta, C.; González, D.; Rodríguez, J.F.; Verdaguer, N. tructural Insights into the Multifunctional Protein VP3 of Birnaviruses. Structure 2008, 16, 29–37.
38. Nicholson, B.L. Use of monoclonal antibodies in identification and characterization of fish viruses. Ann. Rev. Fish Dis. 1993, 3, 241–257.
39. Pedersen, T.; Skjesol, A.; Jørgensen, J.B. VP3, a Structural Protein of Infectious Pancreatic Necrosis Virus, Interacts with RNA-Dependent RNA Polymerase VP1 and with Double-Stranded RNA. J. Virol. 2007, 81, 6652–6663.
40. Tacken, M.G.; Peeters, B.P.; Thomas, A.A.; Rottier, P.J.; Boot, H.J. Infectious bursal disease virus capsid protein VP3 interacts both with VP1, the RNA-dependent RNA polymerase, and with viral double-stranded RNA. J. Virol. 2002, 76, 11301–11311.
41. Bahar, M.W.; Sarin, L.P.; Graham, S.C.; Pang, J.; Bamford, D.H.; Stuart, D.I.; Grimes, J.M. Structure of a VP1-VP3 complex suggests how birnaviruses package the VP1 polymerase. J. Virol. 2013, 87, 3229–3236.
42. Birghan, C.; Mundt, E.; Gorbalenya, A.E. A non-canonical lon proteinase lacking the ATPase domain employs the ser-Lys catalytic dyad to exercise broad control over the life cycle of a double-stranded RNA virus. EMBO J. 2000, 19, 114–123.
43. Lauksund, S.; Greiner-Tollersrud, L.; Chang, C.-J.; Robertsen, B. Infectious pancreatic necrosis virus proteins VP2, VP3, VP4 and VP5 antagonize IFNa1 promoter activation while VP1 induces IFNa1. Virus Res. 2015, 196, 113–121.
44. Magyar, G.; Dobos, P. Expression of infectious pancreatic necrosis virus polyprotein and vp1 in insect cells and the detection of the polyprotein in purified virus. Virology 1994, 198, 437–445.

45. Mundt, E.; Beyer, J.; Muller, H. Identification of a novel viral protein in infectious bursal disease virus-infected cells. J. Gen. Virol. 1995, 76, 437–443.
46. Heppell, J.; Tarrab, T.; Berthiaume, L.; Leeomte, J.; Arella, M. Characterization of the small open reading frame on genome segment A of infectious pancreatic necrosis virus. J. Gen. Virol. 1995, 76, 2091–2096.
47. Skjesol, A.; Skjæveland, I.; Elnæs, M.; Timmerhaus, G.; Fredriksen, B.N.; Jørgensen, S.N.; Krasnov, A.; Jørgensen, J.B. IPNV with high and low virulence: Host immune responses and viral mutations during infection. Virol. J. 2011, 8, 396.
48. Song, H.; Baxter-Roshek, J.L.; Dinman, J.D.; Vakharia, V.N. Efficient expression of the 15-kDa form of infectious pancreatic necrosis virus VP5 by suppression of a UGA codon. Virus Res. 2006, 122, 61–68.
49. Santi, N.; Song, H.; Vakharia, V.N.; Evensen, Ø. Infectious pancreatic necrosis virus VP5 is dispensable for virulence and persistence. J. Virol. 2005, 79, 9206–9216.
50. Hong, J.R.; Gong, H.Y.; Wu, J.L. IPNV VP5, a novel anti-apoptosis gene of the Bcl-2 family, regulates Mcl-1 and viral protein expression. Virology 2002, 295, 217–229.
51. Liu, M.; Vakharia, V.N. Nonstructural Protein of Infectious Bursal Disease Virus Inhibits Apoptosis at the Early Stage of Virus Infection. J. Virol. 2006, 80, 3369–3377.
52. Ortega, C.; Rodríguez, S.; Espinoza, J.C.; Kuznar, J.; Romero, A.; Enríquez, R. Relationship between apoptosis and the BH2 domain sequence of the VP5 peptide of infectious pancreatic necrosis virus. Rev. Mvz. Córdoba 2014, 19, 3990–4002.
53. Hong, J.R.; Lin, T.L.; Hsu, Y.L.; Wu, J.L. Apoptosis precedes necrosis of fish cell line by infectious pancreatic necrosis virus. Virology 1998, 250, 76–84.
54. Ulrich, K.; Wehner, S.; Bekaert, M.; Di Paola, N.; Dilcher, M.; Muir, K.F.; Taggart, J.B.; Matejusova, I.; Weidmann, M. Molecular epidemiological study on Infectious Pancreatic Necrosis Virus isolates from aquafarms in Scotland over three decades. J. Gen. Virol. 2018, 99, 1567–1581.
55. Skjesol, A.; Aamo, T.; Hegseth, M.N.; Robertsen, B.; Jørgensen, J.B. The interplay between infectious pancreatic necrosis virus (IPNV) and the IFN system: IFN signaling is inhibited by IPNV infection. Virus Res. 2009, 143, 53–60.
56. Nouën, C.L.; Toquin, D.; Müller, H.; Raue, R.; Kean, K.M.; Langlois, P.; Cherbonnel, M.; Eterradossi, N. Different Domains of the RNA Polymerase of Infectious Bursal Disease Virus Contribute to Virulence. PLoS ONE 2012, 7, e28064.
57. Ortega, C.; Enríquez, R. Factors associated with cellular infection by the infectious pancreatic necrosis virus (IPNV). Arch. Med. Vet. 2007, 39, 7–18.

58. Kuznar, J.; Soler, M.; Farias, G.; Espinoza, J.C. Attachment and entry of infectious pancreatic necrosis virus (IPNV) into CHSE-214 cells. Arch. Virol. 1995, 140, 1833–1840.
59. Martin, M.C.S.; Villanueva, R.A.; Jashes, M.; Sandino, A.M. Molecular characterization of IPNV RNA replication intermediates during the viral infective cycle. Virus Res. 2009, 144, 344–349.
60. Villanueva, R.A.; Galaz, J.L.; Valdés, J.A.; Jashés, M.M.; Sandino, A.M. Genome assembly and particle maturation of the birnavirus infectious pancreatic necrosis virus. J. Virol. 2004, 78, 13829–13838.
61. Lombardo, E.; Maraver, A.; Espinosa, I.; Fernandez-Arias, A.; Rodriguez, J.F. VP5, the nonstructural polypeptide of infectious bursal disease virus, accumulates within the host plasma membrane and induces cell lysis. Virology 2000, 277, 345–357.
62. Wu, Y.; Hong, L.; Ye, J.; Huang, Z.; Zhou, J. The VP5 protein of infectious bursal disease virus promotes virion release from infected cells and is not involved in cell death. Arch. Virol. 2009, 154, 1873–1882.
63. Marjara, I.S.; Thu, B.J.; Evensen, Ø. Differentially expressed genes following persistent infection with infectious pancreatic necrosis virus in vitro and in vivo. Fish Shellfih Immunol. 2010, 28, 845–853.
64. Julin, K.; Johansen, L.H.; Sommer, A.I.; Jørgense, J.B. Persistent infections with infectious pancreatic necrosis virus (IPNV) of different virulence in Atlantic salmon, Salmo salar L. J. Fish Dis. 2015, 38, 1005–1019.