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REVIEW

The latency related gene of bovine herpesvirus types 1 and 5 and its modulation of cellular processes

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Abstract Bovine herpesvirus type 1 (BoHV-1) and bovine herpesvirus type 5 (BoHV-5) are important pathogens of cattle. The diseases they produce are quite different, with BoHV-5 being more neuropathogenic than BoHV-1 which mainly induces respiratory symptoms. The sequencing of the entire BoHV-5 genome has shown that most of the differences between these viruses are found in the immediate early and LR (latency related) genes. The LR gene is the only viral gene abundantly expressed in latently infected neurons, is essential for viral reactivation and seems to have an anti-apoptotic function which can be observed *in vivo* and *in vitro*. This gene spans two potential ORFs (1 and 2) which can also be found as a fused version, an ORF-E protein encoded within the promoter region and two miRNAs located within the 5’ UTR segment. Most of the essential functions of the LR gene seem to be located within the ORF-2 which has been found to modulate components of cell signaling/cycle pathways. In this review we present a comparative sequence analysis of the LR gene of several BoHV-5 isolates, their differences with the BoHV-1 homologue and the potential impact this may have on its function. The LR gene was found to be highly conserved in all sequenced BoHV-5 strains. ORF-1 shares 60 % homology compared to BoHV-1 whereas the BoHV-5 homologue of ORF-2 is truncated at amino acid 51. Preliminary studies analyzing the emerging transcripts from the BoHV-5 LR gene in infected cells, as well as in stably transfected cells, indicates that their products are, in fact, missing crucial components of the anti-apoptotic function when compared to the BoHV-1 LR gene. In addition these transcripts maintain a region that, similar to what is found in BoHV-1, would produce a miRNA with the potential to recognize a region within the BoHV-5 immediate early gene. All together, these BoHV-5 characteristics suggest that this virus would not possess the same repertoire of latency maintaining functions as BoHV-1. Implications for BoHV-5 neuropathogenic potential are discussed.

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Abbreviations

BoHV-1	Bovine herpesvirus type 1
BoHV-5	Bovine herpesvirus type 5
CNS	Central nervous system
LR	Latency related
ORF	Open reading frame
TG	Trigeminal ganglia
snc	Small non coding

Bovine herpesviruses type 1 and 5 produce different clinical diseases

Bovine herpes virus type 1 (BoHV-1) is the causative agent of “infectious bovine rhinotracheitis (IBR)/ pustular vulvovaginitis (IPV)/ balanopostitis (IPB)” complex [1]. It belongs to family *Herpesviridae*, subfamily *Alphaherpesvirinae*. BoHV-1 may also produce enteritis, abortion and systemic disease [2, 3]. In contrast, BoHV-5 primarily induces neurological disease, including tremors, nystagmus, teeth grinding, circling, ataxia, recumbence, paddling and death. Non suppurative meningoencephalitis is a hallmark of this infection [2, 4–7]. Although there are no

formal reports on the prevalence of BoHV-5, cases of encephalitis are particularly frequent in South America, predominantly in Argentina and Brazil where numerous outbreaks have been described in the last decades, whereas BoHV-1 has a worldwide distribution [4, 8, 9].

Experimental infections with BoHV-5 have demonstrated that it can reach and damage more extensive areas of the bovine central nervous system (CNS) when compared to BoHV-1 [10–13].

Putative BoHV-5 neurovirulence factors have not been extensively studied. BoHV-5 glycoprotein E and envelope protein Us9 sequences have revealed important differences when compared to BoHV-1 and are claimed to play a role in its CNS pathogenicity [14, 15].

BoHV-1 and BoHV-5 genomic differences

BoHV-1 contains a linear double stranded DNA genome of 135 Kb composed of a unique long segment (UL) and a unique short segment (Us) [16]. During lytic infection, transcription of BoHV-1 genes occurs in a cascade fashion and thus, they are denominated immediate-early (IE), early (E) and late (L) genes. IE genes are regulatory genes that stimulate early and late gene transcription. In BoHV-1, IE genes are composed of two transcription units: 1 (IE2.4 and IE 2.9) and 2 (IE 1.7), which express IE genes bICP0, bICP4, and bICP22 [17]. BoHV-5 isolates show 85 % nucleotide identity with BoHV-1 when using hybridization techniques [18] and 82 % overall amino acid identity [19]. These differences are reflected in a characteristic restriction pattern of BoHV-5 which is further divided into several subtypes [20, 21]. The highest similarity to BoHV-1 (>95 % amino acid identity) is found in proteins involved in viral DNA replication and processing (UL5, UL15, UL29, and UL39) and also virion proteins (UL14, UL19, UL48, and US6). Among the least conserved (<75 %) are the homologues of IE proteins bICP0, bICP4, and bICP22, (these three proteins being longer in BoHV-5 than in BoHV-1) and the LR (Latency Related) gene. The LR gene is considered the only BoHV-1 gene expressed during latency [22–24]. The structure of this gene in BoHV-5 diverges markedly from BoHV-1 in both coding and transcriptional regulatory regions.

Characteristics of BoHV-1/BoHV-5 latency/ reactivation processes

Like the human alpha-herpesvirus, herpes simplex virus type 1 (HSV-1), BoHV-1 replicates initially in the nasal and ocular mucosa leading to the characteristic symptoms of the disease [25]. From those tissues and by means of intra-axonal transport, the virus reaches neuronal bodies of the trigeminal ganglia (TG), where it can replicate in some cells. After primary infection, infectious virus is no longer recovered but viral DNA can be detected in TG neurons by *in situ* hybridization [26]. During this stage, there is no transcription of viral genes except for the LR gene. Although many latently infected neurons do not seem to produce infectious virus, a much higher number of neurons are detected in which viral gene expression occurs, suggesting that virus-producing neurons are not common [27].

Upon certain stressful stimuli (dexamethasone mimics stress), latent viral DNA is “reactivated” and infectious viral particles are transported intra-axonally to peripheral tissues. In this stage, apoptosis of inflammatory cells in TG of calves latently infected with BoHV-1 is also observed, and this correlates with reactivation from latency [26].

BoHV-5 also establishes latency in TG of infected calves [12, 28] which has been evaluated by PCR of TG from latently infected calves using glycoprotein C [28] and glycoprotein B [11] specific primers. Although detection of a BoHV-5 LR transcript has not been reported so far it is assumed, by sequence analysis, that a LR-like transcript could eventually be expressed [19]. Similarly to BoHV-1, dexamethasone treatment of BoHV-5 infected bovines induced viral reactivation and shedding [12, 19, 29]. Furthermore, reactivation indicates that BoHV-5 infects the CNS in a more extensive fashion than during the acute stage [11, 12], presenting lesions even in sections of the medulla oblongata. Virus isolation and histopathological analysis of neural tissue sections have also confirmed that viral replication and spread is more extended during reactivation from latency [12]. Unlike BoHV-1, reactivation episodes of BoHV-5 are frequently accompanied by clinical signs [12, 30]. This would suggest that the presence of viral DNA in different areas of the brain might contribute to a more extensive virus distribution in the CNS, favoring recrudescence of the disease during BoHV-5 reactivation.

A model of BoHV-5 infection in rabbit has been developed by several investigators to study neurovirulence [30–33]. These experiments have shown that BoHV-5 replicates and spreads preferentially in the olfactory pathway, similar to what was seen in bovines infected by the same route [12, 13]. However, alternative routes may be also used, like sensory and autonomic fibers of the trigeminal nerve [34]. More recently, the rabbit model has been used to study latency and reactivation from latency [29]. In these experiments it was observed that during reactivation, BoHV-5 can infect more areas of the brain than during the latent stage, in particular the ventrolateral, posterior cerebral cortices and pons medulla. Another important conclusion from the rabbit model experiments has been that not all BoHV-5 strains show the same virulence potential despite having identical LR genes (see below) [34].

The LR gene of BoHV-1 and BoHV-5

During BoHV-1 latency, viral DNA can be detected in latently infected cells but transcription is restricted to only the LR gene which is transcribed in the opposite direction than bICP0 overlapping its 3' end. (Fig 1) [35]. This feature is also present in all the BoHV-5 LR genes reported so far. This transcription in the "opposite" direction led to the original hypothesis that latency occurred by repression of the IE1 transcription unit. In fact, *in vitro* experiments have shown that expression of miRNAs encoded within the BoHV-1 LR gene interfere with bICP0 RNA expression [36]. *In vivo* experiments demonstrated that dexamethasone represses LR promoter activity and LR RNA expression is dramatically reduced 24-48 hours after a reactivation stimulus, suggesting this event is necessary for IE1 transcription and lytic gene expression [27]. In fact, after six hours of dexamethasone treatment BoHV-1 ORF2 and the micro-RNAs are not readily detected (see below) [37].

Insertion of the BoHV-1 LR gene i the HSV-1 LAT locus (CJLAT) restores high levels of spontaneous reactivation in the rabbit eye model and in explant-induced reactivation. Rabbits infected with CJLAT have higher levels of recurrent eye disease (stromal scarring and detached retina) [38]. In a mouse model, CJLAT induced higher frequency of encephalitis compared to the parental LAT null mutant [39]. This suggested that the BHV-1 LR gene and the HSV-1 LAT gene are not functionally identical.

BoHV-1 LR expression is regulated by the LR promoter which contains two neuron-specific regulatory regions designated "neuron specific binding protein region" and "neuron-specific transcriptional activator" (NSB and NSTA, respectively) [40–42]. The few BoHV-5 promoter regions sequenced so far [AY261359/KJ643331.1] show that most of the NSB is missing while the regions 5' to the NSTA (important for expression) are poorly conserved. These observations would suggest a different regulation of the BoHV-5 LR promoter when compared to BoHV-1, particularly in neuronal cells. No functional characterization of this promoter has been described so far.

BoHV-1/5 LR gene products and their modulation of cellular processes

ORFs 1 and 2

The LR gene of BoHV-1 contains two open reading frames (ORF-1 and ORF-2) and two reading frames that lack

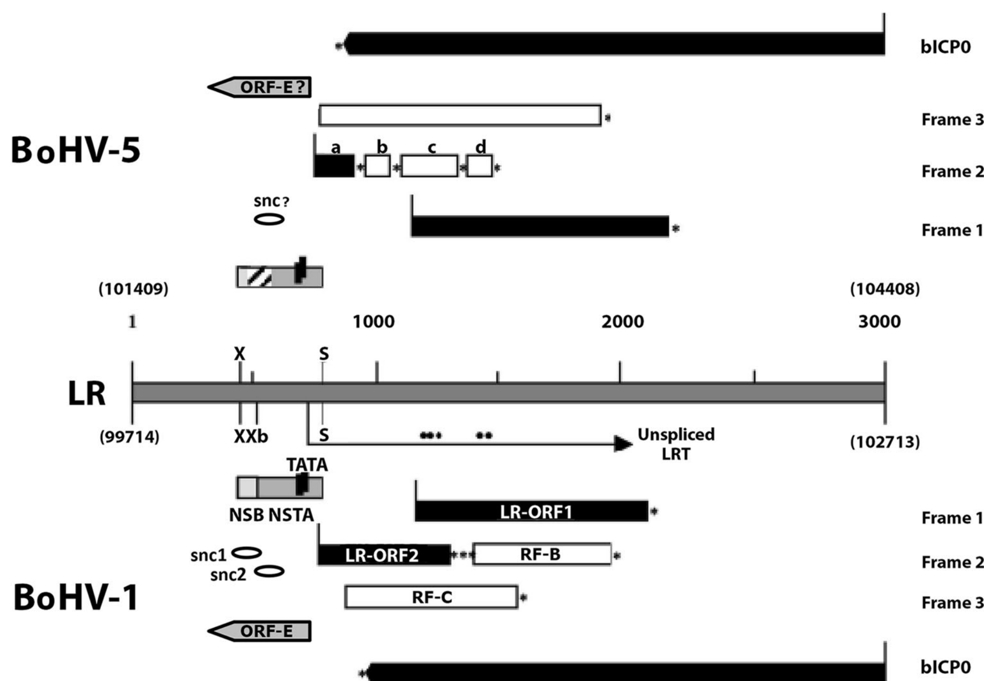


Fig. 1 Comparison of the LR regions of BoHV-5 and BoHV-1. The LR nucleotide positions are given above (BoHV-5) and below (BoHV-1) the central stippled box, and the relative nucleotide positions are in bold (1 to 3,000 bp). Restriction sites are XhoI (X), XbaI (Xb), and SphI (S). The dots above the BoHV-1 LR transcript represent the positions of splice donor signals. Methionine- and non-methionine-initiated ORFs are represented by black and open boxes, respectively. Asterisks indicate the positions of in-frame stop codons. BoHV-1 neuron-specific transcriptional regulatory regions NSB and NSTA are represented by boxes between XhoI and SphI sites; NSB is not present in the BoHV-5 LR promoter (stripped box). Absence of an ORF-E and potential location of the predicted sncRNA are indicated with question marks. Adapted from Delhon [19] with permission

initiating ATGs (reading frame [RF]-B and [RF]-C [35] (Fig 1).

BoHV-1 ORF-2 encodes a putative 181 amino acid protein. Downstream from ORF-2 there is another reading frame without an initiating metionine (Fig 1). In BHV-5, frame 2 is interrupted four times, resulting in reading frames *a* to *d*. Reading frame *a,* which is truncated at 51 amino acids, and reading frame *b* are 82 % and 75 % identical to the corresponding regions in BoHV-1 (Fig 2A).

BoHV-5 ORF-1 shows a 66 % amino acid identity with type 1 version (Fig 2B) (Table 1). All BoHV-5 LR genes recently sequenced (strain Texas/99 [KJ643332.1], Argentina 663 [KJ643331.1], and N569 [KJ643330.1] are basically identical to the original sequence [AY261359] published by Delhon et al (2003) [19] except for minor differences. ORF-2 is identical in all the BoHV-5 strains that have been sequenced (Fig 2A). ORF-1 is also highly conserved in all these BoHV-5 strains except for a few amino acid changes. In the Texas/99 strain there are 18 extra nucleotides not found in the other strains but the overall ORF-1 coding potential is not affected (Fig 2B). The strong sequence conservation among these different BoHV-5 isolates may also be attributed to equally highly conserved bICP0 sequences encoded in the opposite strand of the LR gene. In spite of this the immediate-early gene bICP0 is among the least conserved genes between BoHV-1 and BoHV-5 (70 % amino acid identity). Steiner et al have demonstrated that BoHV-5 bICP0 can fully functionally complement the replication defect caused by deletion of the homologous gene from the BoHV-1 genome [43]. This may be due to the conservation of critical amino acids that maintain the protein conformation necessary for its function.

Numerous preliminary studies on the potential role of the BoHV-1 LR products were performed using a mutant virus containing three stop codons near the beginning of the LR RNA, preventing protein (but not RNA) expression from all three reading frames [44, 45]. This LR mutant also lacked 25 bp from the wild type sequence to prevent reversion to wild type. The LR gene was found to be a contributing factor in the spread of the virus within the TG since the BoHV-1 LR mutant DNA was only detected in neurons during late stages of the lytic infection. In contrast, the wild type virus genome was detected in neuronal and non neuronal cells at all times during the acute stage [46]. Virus spread seems to be supported by LR gene products as well, which directly or indirectly, might inhibit immune recognition in TG. In fact a higher number of inflammatory infiltrates was detected in TG of calves acutely infected

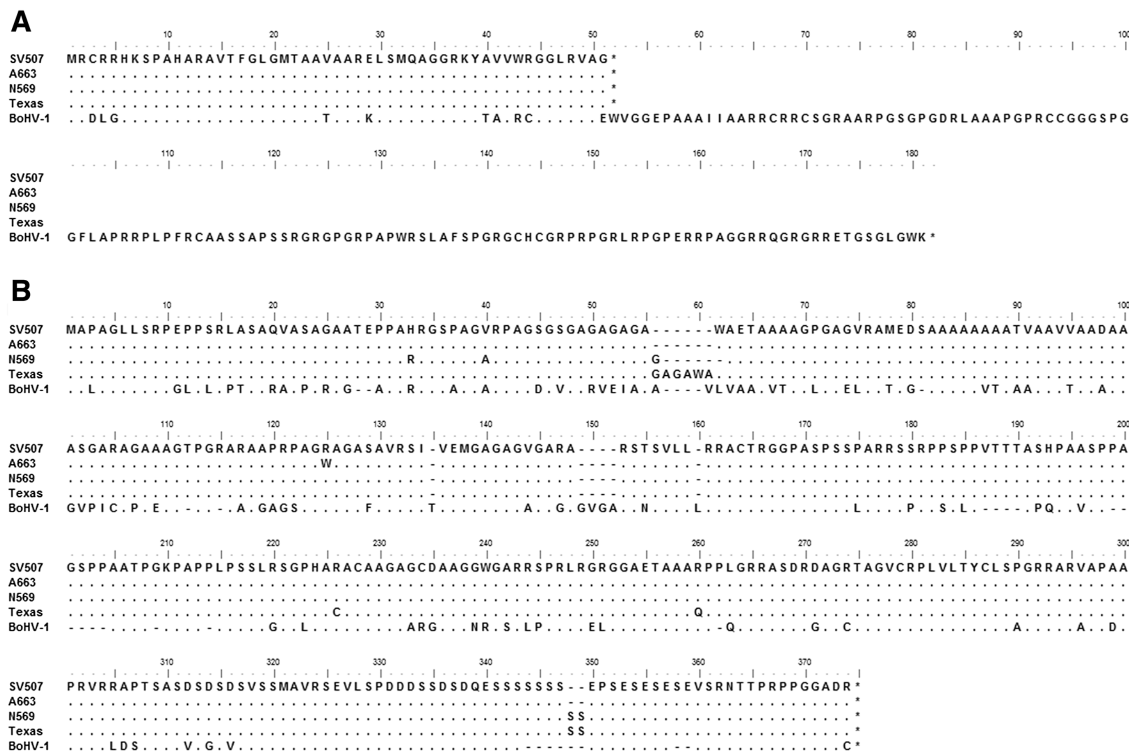


Fig. 2 Bioedit alignments of ORF-2 (a) and ORF-1 (b) aminoacid sequences corresponding to BoHV-5 strains SV507 [GenBank: AY261359], A663 [GenBank: KJ643331.1], N569 [GenBank: KJ643330.1], Texas [GenBank: KJ643332.1], and Cooper BoHV-1[GenBank: M61143] (.) represents identical amino acids with respect to the SV507 sequence. Non conserved amin oacids are shown with the corresponding one letter code. Stop signals are marked with an asterisk, (-) represents gaps

Table 1 Comparison of BoHV-1 and BoHV-5 LR genes

	BoHV-1	Function	BoHV-5*	Ref
ORF-2	181 aa long Present in TG (latency), fused with ORF-1 (7 days) or [RF-B] (15 days) in lytically infected TG Fused with ORF-1 in infected/transfected cell lines	Notch 1 Inhibition 7days cDNA binds apoptotic proteins (Bid/Cdc42) and C/EBP ORF-2 and 15days stimulate neurite formation All forms are antiapoptotic	Only first 51 aa (82 % identity). Conserved in all BoHV-5 sequenced An early stop signal would eliminate any antiapoptotic function Fused with ORF-1 in infected/transfected cell lines; similar to 7 days fusion in BoHV-1; would retain Notch 1 inhibition activity	[17, 35, 38, 53, 54, 61, 63]
ORF-1	344 aa long Alanine-rich region C-terminus serine rich. Potential fusions with other RF during latency in TG	Unknown	360 aa long. 60 % homology with type 1 (conserves alanine rich/serine rich regions) Conserved in most BoHV-5 strains sequenced (slight change in strain Texas)	[35, 38]
Other RF within the LR transcript	Two reading frames that lack initiating ATGs (reading frame [RF]-B and [RF]-C	Unknown	Several reading frames following ORF-2 due to various interruptions. Reading frame *a*, which is truncated at 51 amino acids, and reading frame *b* are 82 and 75 % identical	[35]
ORF-E (within the LR promoter)	Expressed in lytically and latently infected TG, infected cells	Promotes neurite formation in mouse neuroblastoma cells	Potential ORFs can be identified in the BoHV-5 promoter, none similar to ORF-E	[19, 67]
sncRNAs	Two miRNAs transcribed within the 5'UTR	Binds bICP0 reducing its expression Inhibits cell growth?	Bioinformatics analysis predicts at least one miRNA within the same location	[68, 69]
LR promoter	NSB and NSTA	Neuronal Specific Expression	Most of the NSB is missing regions 5' to the NSTA poorly conserved	[27, 36, 44]

C/EBP

* Only structural features are described for BoHV-5, no functional characterization has been reported. NSB: neuron specific binding protein region; NSTA: neuron-specific transcriptional activator

with the BoHV-1 LR mutant when compared to the wild type virus [46]. Bovine kidney cells infected with the LR mutant virus also induced higher levels of beta interferon RNA and interferon response genes [47]. In tonsils of calves acutely infected with this mutant, higher levels of interferon RNA were also described [47].

The BoHV-1 LR gene inhibits apoptosis as well [48] (Table 1). In transiently transfected cells ORF-2 expression is necessary for this function [49]. As mentioned above, BoHV-1 mutants with stop codons near the beginning of ORF-2 that do not express ORF-2, grow less efficiently in certain tissues of infected calves and do not reactivate from latency following dexamethasone treatment [45]. LR mutants also show higher levels of apoptosis in TG neurons of calves when compared to wild type BoHV-1 which correlates with an inhibition of caspase 3 and 9 cleavage in cells expressing the LR gene [50, 51]. In the case of ORF-2, inhibition of apoptosis seems to be in part mediated by its interaction with three cellular transcription factors (C/EBP-alpha, Notch 1 and Notch 3) reducing their trans-activation potential. Through confocal microscopy studies and pull down assays, an interaction of ORF-2 with Notch 1 and 3 has been confirmed [50, 52]. Notch genes (1-4) modulate many neuronal processes [53–55] including the promotion of cell survival by inhibiting apoptosis through protein kinase AKT activation [53–56]. BoHV-1 LR ORF-2 also promotes neurite formation in mouse neuroblastoma cells inhibiting Notch 1/ Notch 3 dependent signaling. This

pathway normally interferes with neuronal differentiation blocking neurite and axon generation. Further studies showed that ORF-2 promoted degradation of Notch 3 whereas Notch 3 RNA and protein levels were increased in TG during reactivation from latency suggesting a role of Notch 3 in this process [57]. However, only Notch 1 had a role transactivating the bICP0 promoter during lytic infection. Overall, these experiments would indicate that BoHV-1 LR ORF-2 has the potential to sequester different transcription factors to promote and maintain latency as well as to increase survival of the infected cell. These effects would lead, in turn, to a higher number of latently infected cells.

Regions of the BoHV-1 LR ORF-2 responsible for inhibiting Notch seem to be different from the ones modulating apoptosis [57]. In fact, as shown by Sinani et al who used transposon insertions in ORF-2, the amino terminal region is relevant for the first function whereas the C-terminus is responsible for inhibiting apoptosis [57]. Since the BoHV-5 LR ORF-2 only shares the first 55 amino acids with the BoHV-1 ORF-2 (Fig 2A) this would suggest that the BoHV-5 ORF-2 could retain the ability to inhibit Notch factors while exhibiting a reduced, or even absent, anti-apoptotic potential. However, the amino terminal region apparently involved in Notch modulation may involve more than just the first 55 amino acids [57]. Both observations point to an LR gene in BoHV-5 with diminished anti-apoptotic activity and reduced inhibition of Notch. All BoHV-1 LR ORF-2-mediated functions seem to be associated with direct binding of the protein to DNA. The nuclear localization signal (NLS) within ORF-2 is identical to the NLS in the transcription factor Sp1, and deletion of amino acids comprising the ORF-2 NLS prevents nuclear localization and reduces binding to DNA [58]. This NLS was found to be crucial for promoting neurite formation in the presence of Notch 1 or Notch 3 [59]. In all BoHV-5 LR ORF-2 sequences analyzed so far no NLS could be identified which could abolish its nuclear localization (unpublished and Fig 2A).

BoHV-1 LR ORF-1 has not been characterized as much as ORF-2. It can be detected in the nucleus and cytoplasm of BoHV-1 infected cells as well as in trigeminal ganglia of latently infected calves [60]. ORF-1 contains an alanine-rich domain with similarities to four cellular transcription factors: histone demethylase, human headcase protein, Brn-3a (a neuronal specific transcription factor) and the homeobox protein Hox-A13 (Fig 2B). In addition, the ORF-1 C-terminus, which is rich in cysteine residues, shows homology to a chromatin-helicase DNA-binding protein 1 (Fig 2B) [60]. None of these regions of similarity have been evaluated functionally, neither is it known whether the ORF-1 plays a role during a productive or latent infection. In the BoHV-5 LR ORF-1 the alanine-rich region is present although it is not identical to the one found in BoHV-1. However, the C-terminus is highly conserved in both ORF-1 suggesting a potential role for this domain.

Fusion proteins between ORF-1 and ORF-2

A fraction of BoHV-1 LR-RNA is polyadenylated (poly+) and alternatively spliced in TG, suggesting this RNA encodes a family of proteins which function during different stages of infection [61, 62]. Differential splicing of the LR transcript can lead to several combinations of these ORFs or RFs. For example, ORF-2 is intact in TG of latently infected calves (60 days post infection) while it can be fused with ORF-1 or RF-B due to alternative splicing of the poly+ LR RNA in lytically infected TG (7 and 15 days respectively) [61]. In addition, different fused ORF-1/ORF-2 transcripts could also be identified in poly-LR RNAs from infected MDBK/transfected Cos7 cells [61, 63]. In fact, an antibody directed against the N terminus of the BoHV-1 LR ORF-2 recognized a 41KDa protein *in vitro* (cell culture) and *in vivo* (trigeminal ganglia) suggesting potential fused derivatives [62, 64, 65]. These observations would suggest that there is a neuronal specific splicing program in the TG compared to other cell types. Moreover, the BoHV-1 LR gene utilizes a GC-AG splicing signal in latently infected calves, similar to the human herpes simplex LAT gene in latently infected mice.

BoHV-1 LR gene products seem to inhibit S phase entry and cell proliferation in part, through association with cyclin-dependent kinase 2 (cdk2) or cdc2/cyclin complexes [64, 66]. This association was observed in transfected human and infected bovine cells but has not been studied *in vivo* so far. However, it is known that BoHV-1 infection of TG induces expression of cyclin A in neurons suggesting that a complex between these proteins and an LR product would inhibit neuronal apoptosis [66].

Coincidentally, an ORF-1/ORF-2 fusion protein encoded by the alternatively spliced BoHV-1 LR-RNA during *in vivo* lytic infection (7 dpi) has been found to inhibit the immediate-early transcription unit 1(IETu1) promoter as well as the herpes simplex virus type 1 ICP0 promoter [63] (Table 1). The 7 dpi cDNA could interact with two pro-apoptotic proteins (Bid and Cdc42) as well as with the CCAAT enhancer-binding protein a (C/EBP-a) repressing viral gene expression and contributing to latency establishment [67]. Recent work has characterized the spliced transcript present in sensory neurons of the TG at the initiation of latency (15 days post infection, 15 dpi) [37]. This transcript could encode a potential protein containing most of the ORF-2 sequences fused with RF-B. When this fused protein was expressed in mouse neuroblastoma cells it stimulated neurite formation and interfered with Notch 3

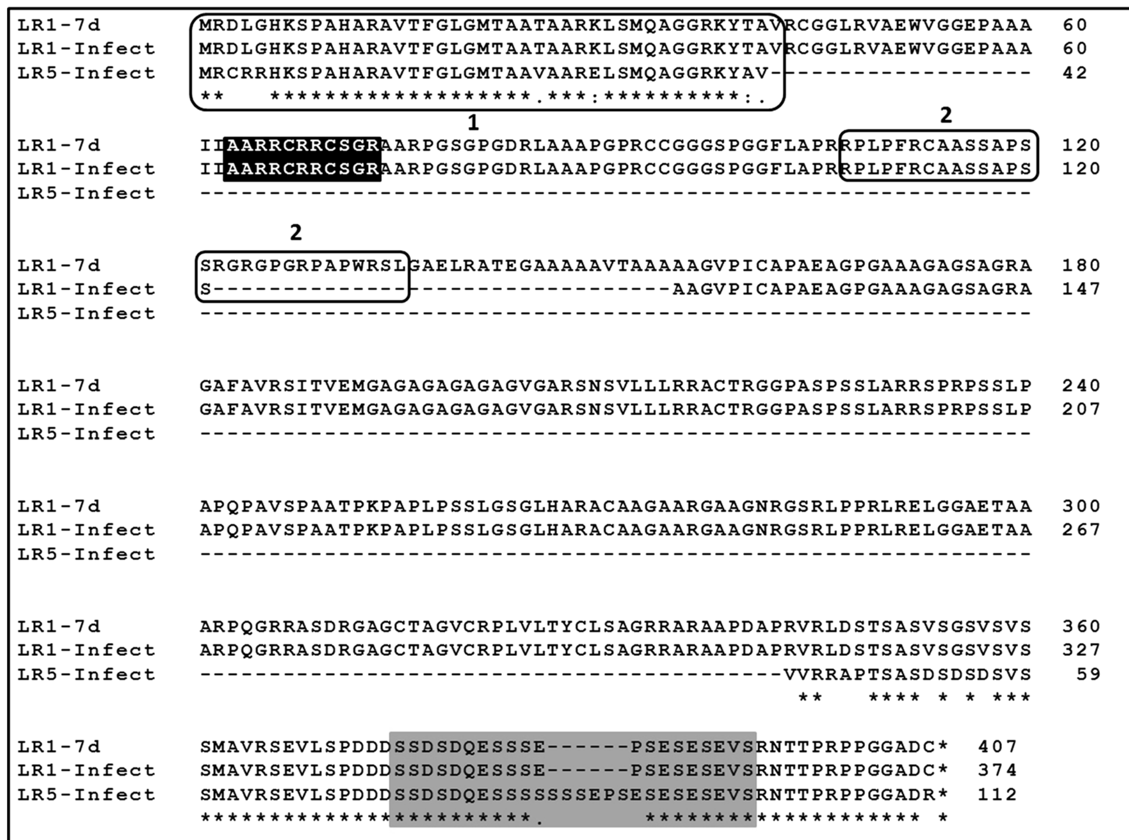


Fig. 3 Analysis of LR fusion products. Amino acid alignment of BoHV-1/BoHV-5 LR fusion products (shown in one letter code) found in BoHV-1 (7 days post infection/MDBK infected cells) and BoHV-5 (infected MDBK cells/stably transfected Neuro2A cells). The alignment was performed with CLUSTAL Omega. (*) corresponds to identical amino acids, (:) corresponds to conserved amino acids, (.) to non-similar amino acids, (-) corresponds to gaps. Box 1, represents the Notch modulation region. Box 2, represents the region with an apoptosis inhibition function. The simile chromatin-helicase DNA-binding protein 1 is shown in grey. The nuclear localization signal is shown in black with white letters

mediated transactivation. In addition this fused product showed greater stability, a feature that would contribute to the establishment of latency [37].

It is not known which spliced versions are produced from the BoHV-5 LR gene during lytic or latent infection. However, our laboratory has identified different spliced versions of the BoHV-5 LR transcript during lytic infection and in stably transfected cells (unpublished and Fig 3). Sequence analysis of the LR ORF-2 shows a consistent stop signal in all BoHV-5 strains (Fig 2A). Thus, if any splicing were present to generate a fusion product between this ORF and other coding sequences this would be located before the stop signal. A cDNA with these characteristics has been detected in MDBK cells infected with BoHV-5 A663 strain. This product removes a 1139bp fragment between 102284 to 103423 bp of the BoHV-5 complete genome [AY261359.1]. This spliced product fuses ORF-2 with ORF-1 keeping the first 55 amino acids of ORF-2 (which are almost identical to the homologous part in BoHV-1) and the 60 amino acids in the C-terminus of ORF-1 including the simile chromatin-helicase DNAse binding domain reported in BoHV-1 [60]. This product, although smaller, has similarities to the one produced by BoHV-1 LR in infected MDBK cells [61]. They also share, with the 7 dpi fused product, a C-terminus rich in cysteine residues (Fig 3).

ORF-E

Over a decade ago, another BoHV-1 LR product denominated ORF-E was described [23]. ORF-E is encoded within the LR promoter and it is antisense to the LR coding sequence. ORF-E protein was reported to promote efficient establishment of latency because of the observation that it could induce neurite-like outgrowth in mouse neuroblastoma cells [67] (Table 1). Consequently, ORF-E protein could enhance restoration of mature neuronal functions following infection. Sequence analysis of the same region in the LR promoter of BoHV-5 SV507/99 and A663 isolates suggests that this ORF-E is not present in BoHV-5. However, other potential ORFs

with no apparent similarities to ORF-E can be detected in both BoHV-5 isolates by bioinformatics tools (data not shown).

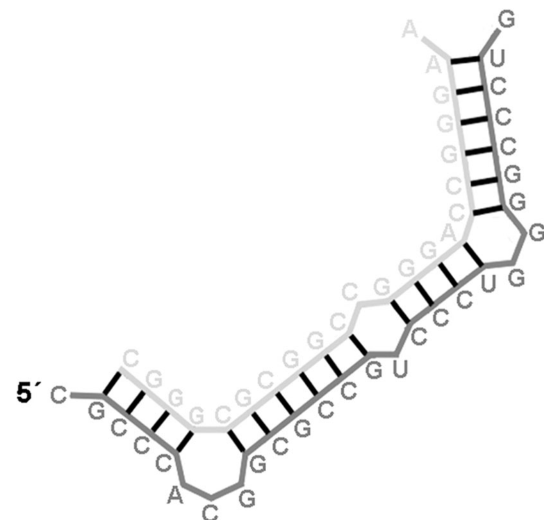
sncRNAs encoded within the LR gene of BoHV-1 and 5

Besides activities mediated by coding sequences, the BoHV-1 LR may also work through non-coding RNA. It has been reported that two sncRNAs encoding two microRNAs (miRNAs) within the 5’UTR region of the BoHV-1 LR gene promote cell survival by interacting with RIG-I (retinoic acid-inducible gene I) and stimulating NF-κB-dependent transcription and beta interferon signaling pathways [68] (Table 1). This correlates with the observation that the BoHV-1 LR mutant that expresses the LR transcript but not translation products induces higher levels of beta interferon RNA and interferon response genes *in vitro* [47]. These miRNAs (or larger small non-coding RNAs containing the miRNA sequences) also reduce bICP0 protein levels in transient transfection assays and have been detected during BoHV-1 latency [69]. These are contained within the same region that was found responsible for a cell growth inhibitory function. Supporting a role of miRNAs, introduction of stop codons in this fragment did not prevent cell growth inhibition [49]. Bioinformatics analysis of the BoHV-5 LR5’ UTR suggests that, similarly to BoHV-1, a potential miRNA is encoded within this region (Fig 4). This viral encoded miRNA matches, almost perfectly, sequences present within the BoHV-5 bICP0 homologue. This observation indicates that the BoHV-5 LR gene could possibly modulate BoHV-5 bICP0 levels through the same mechanism as BoHV-1.

However, a recent work by Tang et al (2014) exploring, by deep sequencing, the miRNAs coding capacity of the BoHV-5 genome found sixteen detectable mature miRNAs during lytic infection. Six out of the sixteen miRNAs were present as two copies in the terminal repeat and terminal repeat regions resulting in seventeen miRNA- encoding loci. From these miRNAs, only one conservative miRNA was shared with BoHV-1, located close to the origin of replication. Different to BoHV-1, no miRNAs were detected in the unique short region or locus within or near the bICP0 and LR genes [70].

Conclusions

BoHV-1 and BoHV-5 have different pathogenic potentials. The LR gene is one of the least conserved between the two viruses. The BoHV-5 LR gene does not have the same coding potential as BoHV-1 LR since it lacks most of ORF-2. Different products from BoHV-1 LR gene are found according to the lytic or latent stage of infection as well as the type of cell in where they are analyzed. Many functions are associated with these products which mainly involve pathways related to cell growth and the inhibition of apoptosis. This role would be more relevant in reactivation than during latency. The anti-apoptotic potential of the BoHV-5 LR has not been addressed so far, but the differences described in this article suggest it could be less efficient at performing this function. Considering their mayor sequence differences additional functions attributed to the LR gene in BoHV-1 may not be present in BoHV-5. Thus, the distinctive LR structure present in BoHV-5 should be evaluated as a potential contributor to the neuropathogenic behavior of this virus.



mfe: -43.8 kcal/mol

Fig. 4 miRNA prediction in the BoHV-5 LR gene. The Bayes-MiRNA gene prediction program (version 1.3) was used to predict mature miRNAs within the LR sequences. The LR sequence of the predicted BoHV-5 miRNA is located between nucleotides 446 to 467 of GenBank KJ643330.1 sequence. The potential of the BoHV-5 LR miRNA to hybridize to bICP0 was analyzed by RNAhybrid, version 2.2. The potential miRNA (light gray) is shown hybridizing with the bICP0 mRNA (red). The 5’ end of the hybridization site (strong gray) corresponds to genomic position 102850 [GenBank: AY261359.1] or bICP0 nucleotide position 1276 to 1249 [GenBank: KJ643330.1]

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Compliance with ethical standards

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Conflict of interest Both authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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