

## Review Article

### The Structure and Function of Jembrana Disease Virus Genome

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#### ABSTRACT

The pathogenic agent of Jembrana disease is a virus which was consequently named Jembrana disease virus (JDV). JDV was definitively defined as a member of the subfamily of lentiviruses of the family of Retroviridae. The genome of JDV is a linear single-stranded RNA, 7732 nucleotides in length. It contains the structural genes *gag*, *pol* and *env* which encode structural and enzymatic proteins that make up infectious viral particles and flanking long terminal repeats (LTR) that characterize all retroviruses. Accessory genes of JDV include *tat*, *rev* and *vif* that are typical of lentiviruses and which are required for the production of regulatory proteins. Products of most accessory genes have been shown to play important roles in viral replication and pathogenesis. The genome of BIV, another bovine lentivirus, is the most related lentivirus to JDV. Though the two bovine lentiviruses have to be considered as two distinct entities as the two viruses are sufficiently different.

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#### INTRODUCTION

Jembrana disease is a bovine disease that affects Bali cattle (*Bos javanicus*). It was first recognized in 1964 as an acute and infectious disease in the Jembrana District of Bali Island in Indonesia (Budiarmo, Hardjosworo, 1976). Due to this report, the disease was therefore designated Jembrana disease. The evidence for a viral aetiology of Jembrana disease was established in 1992. The pathological agent is a lentivirus, consequently called Jembrana disease virus (JDV) (Wilcox et al., 1992). The disease is now endemic through-out parts of Indonesia, including Java, Sumatra (Hartaningsih et al., 1993), and more recently Kalimantan (Borneo), and also in Australia (Chadwick et al., 1998). Jembrana disease constitutes the main concern in Bali cattle industry as it has caused important economic losses due to mortality of cattle. Indeed, during the first observed outbreaks, in 12 months, about 60000 animals died consequently to JDV infection.

The viral nature of the pathogenic agent of Jembrana disease was firstly identified by its size, determined by membrane filtration. It was further established as a member of the family Retroviridae, due to the associated disease, the reverse transcription (RT) activity, the morphological characteristics, the virus maturation by C-type budding through the plasma membrane and into the intracytoplasmic vacuoles, formation of spherical virus particles of 96–124 nm in diameter with smooth membrane which is characteristic of enveloped viruses and frequently with an eccentric nucleoid observed under electron microscopy (Kertayadnya et al., 1993; Wilcox et al., 1992). Furthermore, Western immunoblotting using sera from recovered animals identified several proteins with sizes

ranging from 14 kDa to 100 kDa. Among these antigens, a 24 kDa protein cross-reacted with the 26 kDa capsid protein of bovine immunodeficiency virus (BIV) of the lentivirus subfamily. This analysis allowed to definitively affiliate JDV to the subfamily lentiviruses of the family Retroviridae (Kertayadnya et al., 1993; Wilcox et al., 1995).

The lentivirus affiliation was established by comparison of *pol* gene sequences (Chadwick et al., 1995a). The subfamily of lentiviruses of the Retroviridae family includes many viruses that cause important diseases such as human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2), simian immunodeficiency virus (SIV), equine infectious anaemia virus (EIAV), feline immunodeficiency virus (FIV), caprine arthritis encephalitis virus (CAEV), ovine maedi-visna virus, in addition to JDV and BIV.

#### Genome of JDV

The nucleotide sequence of the JDV genome and the deduced amino acid sequence have now been entirely established. The genome of JDV is a linear single-stranded RNA (GenBank acc. no. NC\_001654; Chadwick et al., 1995b). The genome is 7732 nucleotides in length. Due to this size, it is the smallest lentivirus genome up to date reported. Sequence alignment has also enabled the identification of putative features in JDV genome. It contains the structural genes *gag*, *pol* and *env* which encode structural and enzymatic proteins that make up infectious viral particles and flanking long terminal repeats (LTR) that characterize all retroviruses and finally a number of accessory genes, including *tat*, *rev* and *vif* that are typical of lentiviruses and which are required for the production of

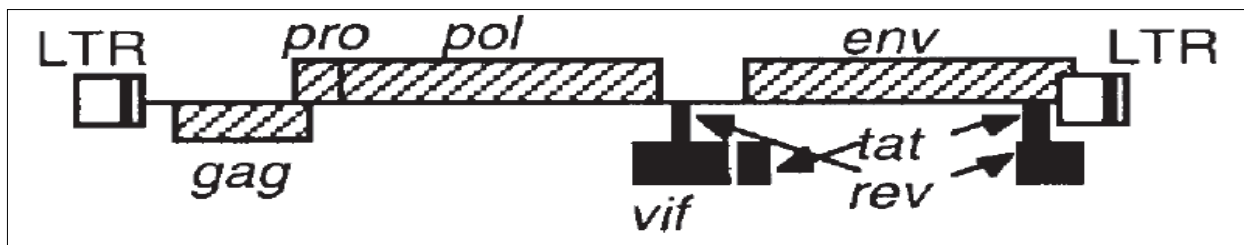


Figure 1: Genomic structure of Jembrana Disease Virus. Shaded box to mark *pol*, *env*, and *gag* genes. Solid box to mark accessory genes

regulatory proteins (Chadwick et al., 1995b). Products of most accessory genes have been shown to play important roles in viral replication and pathogenesis (Kirchhoff et al., 1999).

### Long Terminal Repeats (LTR)

The presence of long terminal repeats (LTRs), flanking both ends of the genome, is one of the characteristics of retroviral genome. The putative JDV LTR is 397 nucleotides long, delineated by the 17 nucleotides primer binding site at the 3'-end and a 18 nucleotides poly-purine tract at the 5'-end. Other features identified were transcription termination signals and 7 nucleotides inverted repeats found at the junction of the chromosomal DNA and the LTRs. Like in other retroviral genomes, JDV LTRs include three regions, successively U3, R and U5. The U3 region, 236 nucleotides long (148 nucleotides shorter than BIV U3), contains enhancer and promoter elements as well as the TATA box which is thought to be essential to the eukaryotic basal promoter but the CAT box, found in most eukaryotic and retroviral promoters is lacking. The downstream U3 region is the putative R region which is 108 nucleotides long and 3 nucleotides shorter than the BIV R region. Within this region, the poly (A) signal and the termination transcription signal are conserved with regards to the BIV ones. The putative 5'-terminus of the viral RNA genome has been identified as the trans-activating response (TAR) region (Chadwick et al., 1995b) to which the trans-activator of transcription (Tat) protein binds for its activity (Carpenter et al., 1992). JDV TAR region has the potential to form a secondary structure, i.e. stem-loop structures, which is more complex than the BIV one. The most downstream region is the U5 region which is 53 nucleotides long and 42 nucleotides shorter than BIV U5 region. The terminal 7 nucleotides repeat (see above) and the tetranucleotide sequence which is supposed to be implicated with transcription termination, are found both in BIV and JDV genomes (Chadwick et al., 1995b).

### Structural Genes

#### Gag Gene

*Gag* gene is one of the three structural genes, *gag*, *pol* and *env* (Chadwick et al., 1995b). The *gag* gene is translated as a single precursor protein which is then cleaved by proteases into three components, i.e. matrix protein (MA) (located at the N-terminal part), capsid protein (CA) which is also the immunodominant virion protein in infected cattle and nucleocapsid protein (NC) (localized at the C-terminal part). The putative JDV *gag* precursor (Pr53<sup>gag</sup>) which is 436 amino acids in length, with a calculated molecular weight of ca. 49 kDa, contains two cleavage sites, instead of five in

BIV. The processing of JDV Pr53<sup>gag</sup> should give rise to (i) MA of 125 amino acids, with a molecular weight estimated to be ca. 14 kDa, (ii) CA (or Cp26), composed of 226 amino acids, with molecular weight of ca. 26 kDa, and NC, 85 amino acids in length and a ca. 9 kDa molecular weight. The three additional BIV short processed products p2L (of 22 amino acids, with a molecular weight of 2.5 kDa), p3 (of 25 amino acids, with a molecular weight of 2.7 kDa) and p2 (of 18 amino acids, with a molecular weight of 1.9 kDa) should be missing in JDV (Chadwick et al., 1995b; Lu et al., 2002). In fact, p2L would be actually missing or incorporated into CA, except for the first three N-terminal amino acids (Lu et al., 2002) and p3 and p2 would be incorporated into NC. At the amino acid level, there is a 62% similarity in *gag* gene products whereas a 75% similarity is found in the capsid protein between JDV and BIV. This strong sequence conservation can so explain the observed serological cross-reactivity between the capsid proteins of the two lentiviruses (Kertayadnya et al., 1993).

#### Gag-pol Gene

Like in most retroviruses and all lentiviruses, JDV *Pol* ORF is translated as a *gag-pol* poly-protein by a -1 ribosomal frame shift. The putative hepta-nucleotide involved in frame shifting mechanism, has also been identified. *Pol* ORF encodes a protease (PR), a reverse transcriptase (RT) and an integrase (IN) proteins (Chadwick et al., 1995b). The predicted *gag-pol* precursor protein of JDV is composed of 1432 amino acids with an estimated molecular weight of 163 kDa. The translation requires a frameshift at nucleotide 1444. The *pol* protein precursor which is translated from the *pol* ORF itself, is 1027 amino acids long and exhibiting a calculated molecular weight of 118 kDa. The ORFs coding for PR, RT, and IN have not yet been determined. The similarity of the *gag* gene is approximately 62% at the amino acid level between JDV and BIV.

#### Env Gene

The product of *env* gene is expressed as a single spliced mRNA. It is translated into an *env* precursor of 781 amino acids in length with an estimated molecular weight of ca. 88 kDa. This precursor is further cleaved at a R-A motif into a surface protein (SU), of 422 amino acids in length and a calculated molecular weight of ca. 47 kDa and a transmembrane protein (TM) of 359 amino acids and a calculated molecular weight of ca. 41 kDa (Kusumawati et al., 2010; Chadwick et al., 1995b). A 31% amino acid identity was found between JDV and BIV *env* gene products overall, with 46% substitutions and 23% mismatches associated with deletions and insertions. JDV TM has a similar size to BIV TM whereas due to a deletion of 471 nucleotides in the

SU region, JDV SU is substantially smaller than its BIV counterpart.

#### Accessory Genes

JDV genome possesses in the central and 3'-terminal domains at least three accessory genes typical of lentivirus genomes, i.e. *vif* (viral infectivity factor), *tat* (trans-activator of transcription), and *rev* (regulator of virus expression). In HIV-1, products of most accessory genes are thought to be functionally implicated in viral replication and pathogenesis. Among them the trans-activator of transcription (Tat) protein which is encoded by *tat* gene, is probably the most important element required for virus life cycle (Arya et al., 1985; Dayton et al., 1986; Huang et al., 1994; Kirchoff et al., 1999; Michael et al., 1995; Westendorp et al., 1995). In JDV, the *tat* gene and its product, i.e. JDV Tat protein (jTat), are the most thoroughly studied among the accessory genes and the proteins they encode. jTat is thought to also play essential functions in virus life cycle and pathogenesis. Furthermore, this protein is a potent trans-activator of transcription, able to activate its own LTR as well as the LTR of other lentiviruses (Calabro et al., 2005; Chen et al 1999b; 2000; Smith et al., 2000; Su et al., 2009; Xie et al., 2004). Its ability to be internalized in noninfected cells and to interfere with cellular gene expression and with cellular cytoskeleton may constitute one of the keys of JDV pathogenesis (Deng et al., 2006; Xuan et al., 2007).

#### Tat Gene

The JDV *tat* gene includes two distantly located open reading frames (ORFs), i.e. coding exon 1 and coding exon 2. The putative jTat protein (jTat), deduced from the nucleotide sequence, is composed of 97 amino acids with a calculated molecular weight of ca. 11 kDa or alternatively of 114 amino acids in length with a calculated molecular weight of ca. 13 kDa. jTat contains a highly conserved cysteine-rich region which presents a 77% identity with that of BIV Tat (bTat). However there is only 8% of sequence identity at the C-terminal domain between jTat and bTat (Chadwick et al., 1995b). Other domains of the 97 amino acids jTat are N-terminal domain, upstream the cysteine-rich region, then successively core domain, basic domain and C-terminal domain (Su et al., 2009).

Like in other lentiviruses, jTat is thought to be produced from multiply spliced mRNAs, due to the presence of numerous splice donors (SD) and splice acceptors (SA), (Chadwick et al., 1995b; Setyaningsih et al., 2008; Stewart et al., 2008). The translation of exon-1, with an ORF of 294 nucleotides in length, is thought to be sufficient to generate a functional jTat (Chen et al 1999b; 2000; Smith et al., 2000; Su et al., 2009) as for e.g. bTat (Liu, et al., 1992). This JDV *tat* ORF will give rise to a deduced jTat of 97 amino acid residues with a calculated molecular weight of ca.11 kDa. The apparent molecular weight of native Tat protein, established by Western blot analysis, is 14 kDa (Setyaningsih et al., 2008). This discrepancy may be due to the presence of positively charged basic residues such as arginine and lysine (Hames, 1998) or post-translational modifications.

#### Rev Gene

The putative *rev* protein is also produced from two coding exons, separately located within the genome. They would generate a 213 amino acids protein, with a calculated molecular weight of ca. 24 kDa (Chadwick et al., 1995b) which proves to be 27 amino acids longer than BIV Rev Protein (Oberste et al., 1993). By using the alternative SD in exon 1, the translation of *rev* gene will result in the production of a 201 amino acids protein, with a calculated molecular weight of ca. 23 kDa. As in most lentiviruses, the greater part of Rev Protein is produced from exon 2. Only 35% amino acid sequence identity is found between JDV and BIV Rev Proteins. Nothing is known, for the time being, on the role of JDV *rev* protein in viral pathogenesis or in other functions.

#### Vif Gene

The putative JDV *vif* gene found in JDV genome has a single potential coding sequence (CDS). Its product is a 197 amino acids protein, with a calculated molecular weight of ca. 23 kDa. Up to now, the function of JDV *vif* protein is not known.

#### The Comparison of Genome of JDV and BIV

BIV is the most related lentivirus to JDV. The genome of BIV is a linear single-stranded RNA of 8960 nucleotides in length (GenBank acc. no. M32690 [BIV127]; Garvey et al., 1990). The comparison of genome of BIV and JDV has verified some differences between the two genomes (Chadwick et al., 1995b). The JDV genome is much shorter and strikingly many deletions and insertions have been found relative to that of BIV. Despite the error-prone nature of lentiviral reverse transcriptases (RT) and the genomic variability *in vivo*, it has never been observed before that two closely related lentiviruses feature so many deletions and insertions rather than substitutions relative to each other. Despite being 750 nucleotides shorter, the genomic organization of JDV genome is almost identical to that of BIV. A high degree of nucleotide and amino acid homology can be observed throughout the two genomes. The presence of small open reading frames (ORFs) in central and 3'-terminal domains also shows that the JDV genomic organization is typical to lentiviruses. Relative to BIV genome, JDV genome shows 27 deletions, totalling 897 nucleotides, and 17 insertions, totalling 147 nucleotides, and resulting so in 750 nucleotides shorter genome (Chadwick et al., 1995b). Comparison of 9 partial *pol* (polymerase) gene sequences from known lentiviruses has shown that the closest alignment of JDV *pol* gene sequence is found with that of BIV, indicating a 74% identity at the nucleotide level and 76% at the level of amino acid, and importantly with no deletions or insertions. This analysis definitively confirms that JDV is actually a lentivirus. On the basis of partial *pol* gene sequence, JDV is the most related to but distinguishable from BIV. The phylogenetic analysis also estimates that the genetic distance between JDV and BIV is comparatively shorter than that between the two human immunodeficiency viruses, HIV-1 and HIV-2 (Chadwick et al., 1995a).

## Concluding Remarks

Although BIV, another bovine lentivirus, is genetically and antigenically the most related to JDV, the two bovine lentiviruses have to be considered as two distinct entities as the two viruses are sufficiently diverging, due to important deletions and insertions (Chadwick et al., 1995b). Another reason to consider the two viruses as separate entities is the fact that the associated disease syndromes radically differ each from the other. In contrast to the mild disease syndrome associated with most lentiviruses, including BIV, JDV infection is associated with an acute, severe and often lethal disease. This difference may wholly or partially result from differences found in their genome and may explain the markedly increased level of viral expression and *in vivo* pathogenicity of JDV. This may result from more efficient enhancer elements, changes in *rev* gene products or a much more potent Tat protein.

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