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The molecular biology of bovine immunodeficiency virus: a comparison with other lentiviruses

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Abstract

Bovine immunodeficiency virus (BIV) was first isolated in 1969 from a cow, R-29, with a wasting syndrome. The virus isolated induced the formation of syncytia in cell cultures and was structurally similar to maedi-visna virus. Twenty years later, it was demonstrated that the bovine R-29 isolate was indeed a lentivirus with striking similarity to the human immunodeficiency virus. Like other lentiviruses, BIV has a complex genomic structure characterized by the presence of several regulatory/accessory genes that encode proteins, some of which are involved in the regulation of virus gene expression. This manuscript aims to review biological and, more particularly, molecular aspects of BIV, with emphasis on regulatory/accessory viral genes/proteins, in comparison with those of other lentiviruses.

Keywords: molecular biology; bovine immunodeficiency virus; lentiviruses

Introduction

Retroviruses represent viruses which infect or can be found in animal species covering a large taxonomic range. All retroviruses have the common property of a requirement for synthesizing a DNA copy of their RNA genome by reverse transcriptase during their replicative life cycle (Goff, 2001). Lentiviruses belong to a unique genus of retroviruses which share structural, genetic, biological and/or pathological properties. Lentiviruses include maedi-visna virus (MVV) in sheep, caprine arthritis-encephalitis virus (CAEV), equine infectious anemia virus (EIAV), Jembrana disease virus (JDV) in cattle, bovine immunodeficiency virus (BIV), feline immunodeficiency virus (FIV), simian immunodeficiency virus (SIV) and human immunodeficiency virus (HIV) (Table 1). Lentiviruses, which are not oncogenic, induce slow, chronic and degenerative pathological changes in infected hosts, often associated with the development of immune-mediated lesions (Desrosiers, 2001). All lentiviruses infect monocyte/macrophage cells. Moreover, FIV, SIV and HIV infect T cells and, consequently, are mainly associated with clinical signs of immunodeficiency in the infected hosts (Gonda *et al.*, 1987; Chen *et al.*, 1999b; Lechner *et al.*, 1997; Turelli *et al.*, 1997; Agnarsdóttir *et al.*, 2000). In contrast to the other retroviruses, lentiviruses may replicate in non-dividing cells. In addition, the lentivirus genome offers a complex structure including several regulatory/accessory genes that encode proteins, some of which are involved in the regulation of virus gene expression.

HIV, the causative agent of the human acquired immune deficiency syndrome (AIDS), is the most studied lentivirus. Although the macaque appears to be the gold standard for AIDS as an animal model, no single virus–animal model is sufficient for all aspects of HIV and AIDS research. Therefore, other lentiviruses, including BIV, may constitute alternative surrogate animal models for certain aspects of HIV research. In addition, conducting basic research on new aspects of lentiviruses is important not only for the virus itself, but also for the entire lentivirus/retrovirus field. This review focuses on the biological and molecular properties of BIV, with emphasis on regulatory/accessory viral genes/proteins, in comparison with those of other lentiviruses.

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Table 1 Clinical manifestations of known lentiviruses

Lentivirus	Host	Cell tropism	Clinical disorders
Maedi-visna virus (MVV)	Sheep	Macrophages	Encephalitis, pneumonia, lymphadenopathy, paralysis, opportunistic infections
Caprine arthritis-encephalitis virus (CAEV)	Goat	Macrophages	Arthritis, encephalitis, paralysis
Equine infectious anemia virus (EIAV)	Horse	Macrophages	Hemolytic anemia, lymphoproliferation, glomerulonephritis, encephalopathy
Bovine immune deficiency virus (BIV)	Cattle	Macrophages	Lymphocytosis, lymphadenopathy, central nervous system lesions, weakness, emaciation
Jembrana disease virus (JDV)	Balinese cattle	Macrophages	Fever, lethargy, anorexia and enlargement of the lymph nodes
Feline immunodeficiency virus (FIV)	Cat	T lymphocytes	Immunodeficiency, lymphadenopathy, leucopenia, anemia, opportunistic infections
Simian immunodeficiency (SIV)	Primates	T lymphocytes	Immunodeficiency, neuropathology, opportunistic infections in rhesus macaque
Human immunodeficiency virus (HIV)	Human	T lymphocytes	Immunodeficiency, lymphadenopathy, opportunistic infections, encephalopathy, Kaposi's sarcoma

Historical perspectives on bovine lentiviruses

In 1969, Dr Cameron Seger, a veterinary practitioner in the state of Louisiana, observed progressive deterioration in the physical condition of an 8-year-old pregnant dairy cow called R-29. The clinical signs observed in that animal included elevated white blood cell counts, lymphoadenopathy, evidence of central nervous system lesions, progressive weakness and emaciation suggesting bovine leukosis (Malmquist *et al.*, 1969). When the virus was inoculated into colostrum-deprived young calves, these animals developed lymphoadenopathic lesions and leukocytosis which persisted for several months (Van Der Maaten *et al.*, 1972). Histopathological studies revealed follicular hyperplasia in the lymph nodes and the presence of infiltrating mononuclear cells within the brain tissues of these calves (Van Der Maaten *et al.*, 1972). The virus was first designated 'bovine visna-like virus' and remained unstudied until HIV was discovered in 1983 (Barre-Sinoussi *et al.*, 1983). Then, Gonda *et al.* (1987) demonstrated by molecular and immunological techniques that the bovine R-29 isolate was indeed a lentivirus with striking similarity to HIV. Consequently, the designations of 'bovine immunodeficiency-like virus' and, thereafter, 'bovine immunodeficiency virus' were used.

Most information on the molecular biology of BIV derived from the work of Braun *et al.* (1988) and Garvey *et al.* (1990), who generated and characterized two infectious cDNA clones, called BIV 106 and BIV 127, from the R-29 isolate of BIV. Thereafter, Suarez *et al.* (1993) isolated two additional BIV field strains, termed FL491 and FL112, associated with the development of leukocytosis. Nevertheless, most pathological, serological and molecular biology information has been obtained from studies with the original BIV R-29 isolate. Another viral isolate, JDV, has been described. JDV is a bovine lentivirus genetically and antigenically related to BIV. It causes an unusual clinical disease in Balinese cattle (*Bos javanicus*) characterized by signs of fever, lethargy, anorexia and enlargement of the lymph nodes, and death of a significant number of infected animals within 1–2 weeks after infection (Chadwick *et al.*, 1995; Wilcox *et al.*, 1995; Wareing *et al.*, 1999).

Seroprevalence and clinical/pathological features of BIV

BIV is distributed world-wide, as it has been serologically detected in Europe, Asia, Australia, New Zealand and North America (Cockerell *et al.*, 1992; Muluneh, 1994; StCyr Coats *et al.*, 1994; Hirai *et al.*, 1996b; Polack *et al.*, 1996; Cavirani *et al.*, 1998; Meas *et al.*, 1998, 2000a, b; Cho *et al.*, 1999; Burkala *et al.*, 1999; Scobie *et al.*, 2001). BIV is pathologically more related to lentiviruses associated with chronic inflammatory diseases (CAEV and EIAV) rather than those associated with severe immunodeficiency (HIV, FIV and SIV). As most infections occur with no evidence of clinical disease, the scope of BIV infection in cattle has never been clearly established. However, BIV does replicate in monocyte/macrophage cells, with a possible dysfunction of the immune system (Carpenter *et al.*, 1992; Onuma *et al.*, 1992; Zhang *et al.*, 1997a). Hence, several secondary conditions, including mastitis, pododermatitis and other bacterial diseases, are associated with BIV infection, thus suggesting a possible impact on dairy herd productivity and general health (McNab *et al.*, 1994; Jacobs *et al.*, 1995). In addition, an association between BIV infection and the development of the bovine paraplegic syndrome was suggested (Walder *et al.*, 1995).

Although BIV infection occurs generally in the absence of clinical signs of disease, several factors may

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influence the development of apparent clinical infection. They include stress stimuli such as exposure to extreme temperatures, parturition and lactation (Snider *et al.*, 1997). Genetic predispositions of the natural host to respond to pathogens or infections by other viruses might also influence the course of BIV infection. For the latter, it is noteworthy that bovine leukemia virus (BLV), bovine syncytial virus (BSV), and bovine herpes virus (BHV) can activate BIV gene expression *in vitro* (Geng *et al.*, 1992; Pallansch *et al.*, 1992). Whether these viruses activate BIV gene expression *in vivo* has yet to be determined. Nonetheless, reduction of *in vitro* lymphoproliferative responses to specific antigens or to mitogens (phytohemagglutinin, concanavalin A and pokeweed mitogen) was demonstrated with mononuclear cells isolated from cattle or sheep experimentally exposed to BIV, or to both BIV and BLV (Martin *et al.*, 1991; Hirai *et al.*, 1996a; Zhang *et al.*, 1997a).

Transmission, cell tropism and host range of BIV

BIV can be transmitted vertically *in utero* or horizontally by the exchange of body fluids, including blood and colostrum (Nash *et al.*, 1995b; Moore *et al.*, 1996; Snider *et al.*, 1997; Venables *et al.*, 1997; Munro *et al.*, 1998; Meas *et al.*, 2002; Moody *et al.*, 2002). *In vivo*, BIV DNA was detected in a large variety of bovine tissues, including brain, lungs, lymph nodes, spleen, peripheral blood mononuclear cells (PBMC) and semen of infected animals (Gonda *et al.*, 1990; Pifat *et al.*, 1992; Baron *et al.*, 1995, 1998; Nash *et al.*, 1995a; Zhang *et al.*, 1997b; Gradil *et al.*, 1999). BIV replicated *in vitro* in a wide variety of cells, such as bovine, ovine, rabbit and canine cells (Bouillant *et al.*, 1989; Gonda *et al.*, 1990; Zhang *et al.*, 1997b), but not human cells (Kashanchi *et al.*, 1991; Whetstone *et al.*, 1992). BIV induced a cytopathic effect characterized by the formation of syncytia in permissive cells. Moreover, virus gene expression varies widely according to the cell type, suggesting that specific cellular factors are required for productive infection (Fong *et al.*, 1997; Kempster *et al.*, 2002).

The discovery of the causative agent of human AIDS led to the development of animal models for HIV research. Similarly, animal models were also developed for BIV research. For instance, goats and sheep experimentally infected with BIV develop a virus-specific humoral response without the development of clinical disease (Whetstone *et al.*, 1991; Smith and Jacobs, 1993; Jacobs *et al.*, 1994; Smith *et al.*, 1994; Hirai *et al.*, 1996a). In 1992, two studies conducted independently demonstrated that persistent infection can be established in white New Zealand rabbits inoculated with BIV R-29 (Pifat *et al.*, 1992; Van Der Maaten and Whetstone, 1992). In these infected rabbits, BIV was rescued from PBMC and spleen, lymph nodes and brain by the cocultivation method (Pifat *et al.*, 1992). Moreover, a rapid and long-lasting virus-specific humoral immune response was observed in rabbits infected with BIV (Abed *et al.*, 1999; Abed and Archambault, 2000). No clinical symptoms were observed in BIV-infected animals during all these studies. In contrast, other studies (Kalvatchev *et al.*, 1995, 1998, 2000; Walder *et al.*, 2001) showed the development of clinical signs of disease (anorexia, weight loss, muscular wasting, diarrhea, hypoalgesia, torticollis, recurrent T- and B-cell dysfunctions, lymphadenopathy and splenomegaly) in several rabbits infected with BIV R-29, whereas the others remained asymptomatic.

Morphology of BIV

BIV is an enveloped virus 120–130 nm in diameter (Fig. 1). The bilayer viral envelope, which contains the viral surface (SU) gp100 and transmembrane (TM) gp45 proteins, surrounds conical-shaped capsid (CA) and nucleocapsid (NC) structures protecting the BIV genome. The genome is composed of a capped and polyadenylated diploid RNA 8482 nucleotides in length that is closely associated with viral proteins p7 and p13 (Gonda *et al.*, 1994).

The replication life cycle of BIV

The BIV replication life cycle is similar to that of other retroviruses (Fig. 2). Viral infection is initiated when the

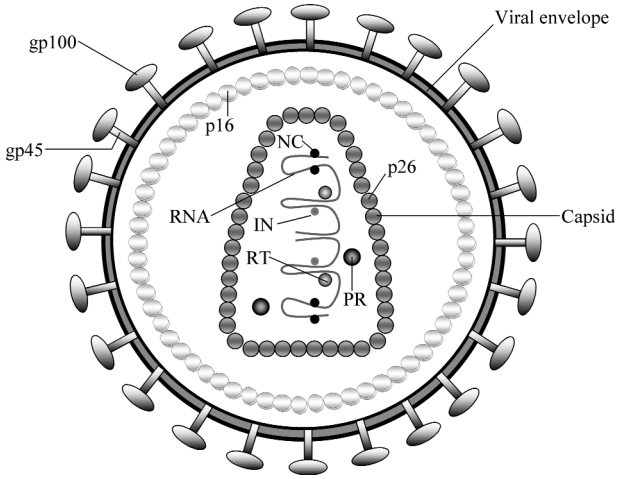

Fig. 1. Schematic morphology of bovine immunodeficiency virus (BIV). The viral envelope is composed of the surface (SU) gp100 and transmembrane (TM) gp45 glycoprotein, and p16 protein forms the viral matrix. The cone-shaped structure typical of lentiviruses is composed of the capsid protein p26 and surrounds the viral enzyme proteins integrase (IN), protease (PR) and reverse transcriptase (RT) and the genomic RNA, which is protected by the nucleocapsid (NC).

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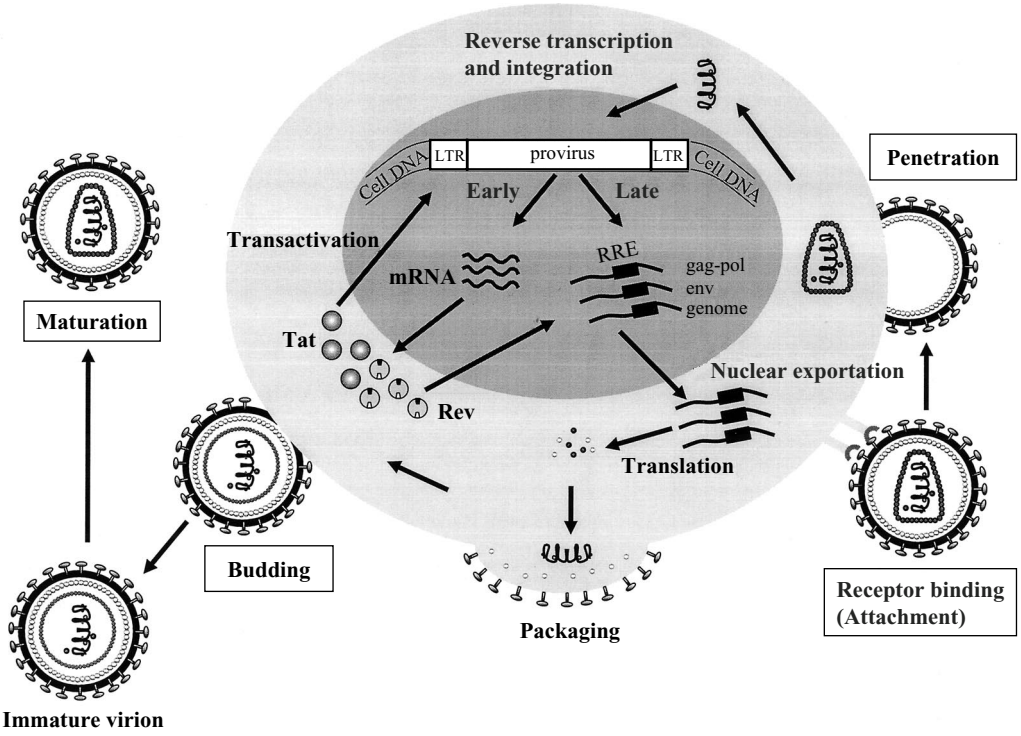

Fig. 2. The replication life cycle of bovine immunodeficiency virus (BIV).

BIV SU protein binds to target cells. This interaction promotes a conformational change that exposes the hydrophobic domain of the viral TM protein, resulting in fusion of the viral envelope with the membrane of the infected cell. This fusion facilitates the entry of the virus within the cell, and is followed by the release of the viral capsid into the cytoplasm (Sommerfelt, 1999). Although the cell receptor for BIV has yet to be determined, it is suggested that BIV could bind to CCR5, a molecule of the β-chemokine receptor family (Wright *et al.*, 2002). CCR5 acts as a co-receptor for the infectivity of certain strains of HIV showing a tropism for cells of the monocyte/macrophage lineage (Alkhatib *et al.*, 1996; Wu *et al.*, 1997).

The uncoating event releases the genomic RNA into the cytoplasm of the infected cell, where it is reverse-transcribed by the viral reverse transcriptase encoded by the *pol* gene into double-stranded DNA (also known as the provirus DNA). Thereafter, the provirus DNA integrates into the host cell genome through the action of the viral integrase. The provirus DNA can remain silent, or, upon appropriate stimuli, serves as a DNA template for the synthesis of new viral RNAs.

BIV gene expression is characterized by five viral mRNAs that are 8.5, 4.1, 3.8, 1.7 and 1.4 kb in length (Oberste *et al.*, 1991). In early events, non-structural regulatory *tat* and *rev* genes are translated from the small multiply spliced viral transcripts of 1.7 and 1.4 kb, respectively. Thereafter, the Tat protein migrates to the nucleus to enhance expression of all genes of BIV, whereas the Rev protein is involved in the transport from the nucleus to the cytoplasm of the late singly spliced or unspliced viral RNAs. The capsid protein (derived from the Gag precursor) and the Gag-Pol enzyme precursor (see below) are translated from the full-length transcript of 8.5 kb. Translation of the singly spliced transcript of 3.8 kb results in the production of the envelope (Env) protein, whereas the singly spliced transcript of 4.1 kb would produce a putative protein, termed viral infectivity factor (Vif). The Env protein, as for the Gag and Gag-Pol precursors, migrates to the cell plasma membrane, where the genomic RNA is packaged during the budding of morphologically immature virions through the plasma membrane of infected cells. The cone-shaped morphology, typical of mature lentiviruses, arises after Gag and Gag-Pol precursor cleavage by the viral protease. Then, the newly produced viral particles can initiate a novel infectious cycle by infecting surrounding non-infected cells.

BIV provirus genomic organization and regulation of viral gene expression

BIV has the most complex genome of the non-primate lentiviruses (Fig. 3). The BIV proviral DNA is 8960 nucleotides long and resembles other retroviruses with the typical 5′–3′ *gag*, *pol* and *env* gene arrangement. These genes encode viral structural proteins, namely the *gag*-encoded capsid p26 protein (p26) and the above-

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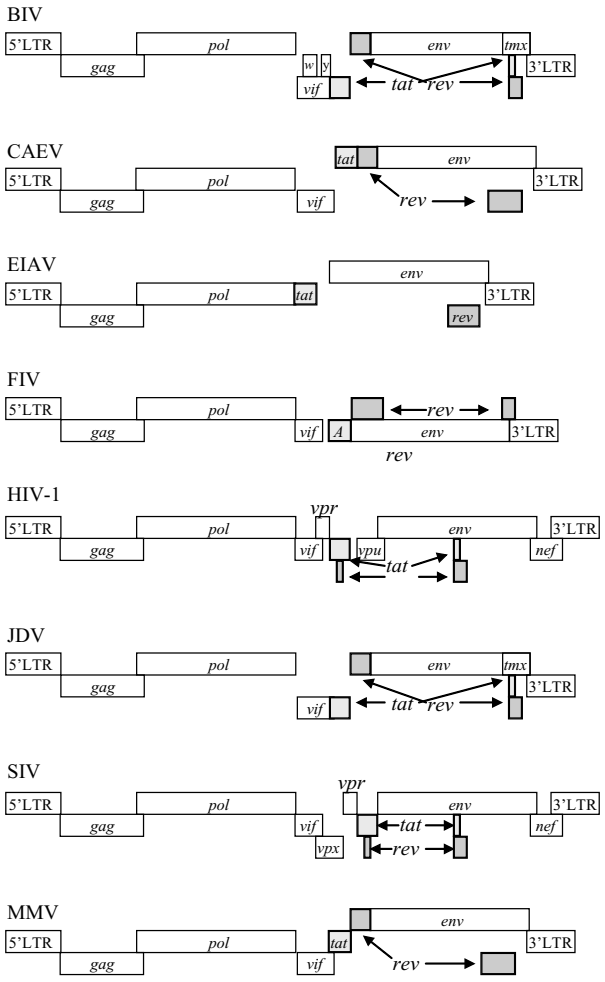

Fig. 3. The proviral genome of various lentiviruses, including bovine immunodeficiency virus (BIV), caprine arthritis–encephalitis virus (CAEV), equine infectious anemia virus (EIAV), feline immunodeficiency virus (FIV), human immunodeficiency virus type 1 (HIV-1), Jembrana disease virus (JDV), simian immunodeficiency virus (SIV) and maedi-visna virus (MMV) with the typical retroviral *gag, pol* and *env* gene organization, and the presence of long terminal repeats (LTRs) and regulatory/accessory genes identified in BIV as *vif, tat, rev, vpy* (*y*), *vpw* (*w*) and *tmx*.

mentioned *env*-encoded SU and TM proteins, which are highly immunogenic (Abed *et al.*, 1999; Abed and Archambault, 2000), and enzymes such as the reverse transcriptase, integrase and protease necessary for the synthesis and integration of the provirus DNA into the host cell genome, and for viral polyprotein processing, respectively (Gonda *et al.*, 1994). The proviral genome has two flanking sequences, called long terminal repeats (LTRs), used in the regulation of viral replication and gene expression (Gonda *et al.*, 1994). The LTRs derive, through reverse transcription, from the redundant R regions and the unique 5′ (U5) and 3′ (U3) present at the termini of the genomic RNA. The BIV genome also contains six non-structural regulatory/accessory protein-encoding genes between or overlapping the *pol* and *env* open reading frames (ORFs). The non-structural ORFs are designated *vif*, *tat* (transactivator factor of transcription), *rev* (regulator of virus expression), *vpw*, *vpy* and *tmx*. The *vpw*, *vpy* and *tmx* genes are unique to the bovine lentivirus. Although *tat*, *rev* and *tmx* messages were detected in BIV-infected cells, only the Tat and Rev proteins were shown to have regulatory functions in virus expression in BIV (Gonda *et al.*, 1994). It is noteworthy that the mRNA transcript of *tat*, as for the *tmx* transcript, is detected by reverse transcription–polymerase chain reaction from PBMC of BIV-infected cattle (Baron *et al.*, 1995).

Lentiviruses use a variety of *cis*-acting signals and viral regulatory/accessory proteins to modulate various aspects of their replication and infectivity. BIV gene expression is highly dependent on interactions of host cell transcriptional factors with various *cis*-regulatory elements located within the LTRs. The BIV LTRs, 587 to 589 nucleotides in length, contain nucleic acid motifs necessary for the initiation, enhancement and termination of viral transcription. The *cis*-acting elements located within the U3 region of the LTR include nucleic acid binding sites for the NF-κB, GRE, AP-4, AP-1, CAAT, ATF/CRE and Sp1 transcription factors, and for the core enhancer. All these elements are essential for BIV LTR functional activity (Fong *et al.*, 1995). The BIV U3 region contains only a single nucleic acid motif for most of the transcription factor binding sites as compared to virulent lentiviruses such as HIV but also three nucleic acid motifs associated with the Sp1 transcription factor binding site. All BIV transcripts are initiated at the +1 position of the 5′ LTR R region. The R region is also responsible for the synthesis of the transactivation responsive (TAR) element, a hairpin RNA sequence located at the 5′ termini of all viral RNA transcripts. Finally, all viral RNAs contain an untranslated common leader sequence at their respective 5′ terminal and a polyadenylated tail at the 3′ terminal which is transcribed from the 3′ LTR.

The BIV *gag, pol* and *env* gene-encoded products

The major structural genes of BIV (*gag, pol* and *env*) encode polyprotein precursors to generate structural (Gag and Env) and viral enzyme (reverse transcriptase, integrase and protease) proteins. The *gag* (for *g*roup *a*ntigen-associated *g*ene) gene, located downstream from the 5' LTR, encodes the Gag (Gag Pr53) precursor protein. It also encodes a portion of the Gag-Pol (Pr170) precursor. Gag Pr53 (with a molecular mass of 53 kDa) is processed, during viral maturation, by the viral protease into matrix (MA) p16, capsid (CA) p26, nucleocapsid (NC) p13, and three small proteins p2L, p3 and p2 (Rasmussen *et al.*, 1990; Battles *et al.*, 1992; Tobin *et al.*, 1994). The order of cleavage products in

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BIV Gag Pr53 is as follows: NH2-MA-p2L-CA-p3-NC-p2-COOH (Tobin *et al.*, 1994). In addition, Gag Pr53 contains, between the CA and NC regions, a short spacer sequence shown to be essential for BIV assembly (Guo *et al.*, 2004). Following virus maturation, MA protein remains associated with the inner side of the viral envelope, whereas CA forms a conical shell surrounding the viral RNA-NC complex (Tobin *et al.*, 1994). In contrast to other retroviruses, BIV MA is not myristylated (Tobin *et al.*, 1994). Also, BIV CA protein contains major epitopes for the host's virus-specific antibody response (Whetstone *et al.*, 1991; Atkinson *et al.*, 1992). In addition, antisera specific to BIV CA and NC proteins show cross-reactivity to analogous HIV-1 and JDV proteins (Gonda *et al.*, 1987; Lu *et al.*, 2002). Finally, an epitope located in a region of Gag Pr53 overlapping MA and p2L is used to distinguish BIV from JDV infection (Lu *et al.*, 2002). The roles of p2L, p3 and p2 in the BIV replication life cycle have yet to be determined.

The *pol* gene partially overlaps the Gag-encoding sequence. During *gag* translation, a –1 frameshift event occurs near the 3′ terminus of the *gag* gene by a mechanism that is poorly understood (Battles *et al.*, 1992). The resulting translation product is a polyprotein of 170 kDa, called Pr170 precursor (with a molecular mass of 170 kDa). Pr170 is then processed by cellular proteases into the protease (PR), reverse transcriptase (RT) and integrase (IN). RT with both polymerase and RNase activity with Mg^{2+} cofactor is responsible for the proviral DNA synthesis from the viral genomic RNA. PR function is to cleave Gag Pr53, whereas IN promotes the integration of the provirus DNA into the host cell DNA (Clements and Zink, 1996; Hindmarsh and Leis, 1999).

The *env* gene, located in the 3′ region of the BIV genome, encodes the highly glycosylated Env precursor gPr145 (Gonda *et al.*, 1994). gPr145 (with a molecular mass of 145 kDa) is further processed by cellular proteases into the SU and TM proteins (Rasmussen *et al.*, 1992). The SU protein is associated with the extracellular domain of TM through electrostatic binding in the virion, whereas TM spans the viral envelope through a highly hydrophobic domain required to anchor the SU–TM complex to the viral envelope. As for the other lentiviruses, the SU protein determines cell tropism of the virus through its attachment to cell receptors, whereas TM promotes the fusion of viral and cellular membranes. TM is also responsible for the formation of syncytia in BIV-infected cells *in vitro* (Chirmule and Pahwa, 1996).

A key feature of BIV infection is the induced antibody immune response. Similar to that reported with HIV p24 capsid protein in the course of HIV infection in the human (Gaines *et al.*, 1987), immune reactivity associated with the BIV major capsid protein p26 appears early in animals experimentally exposed to BIV (Whetstone *et al.*, 1990, 1991). However, this immune reactivity has been shown to decrease to undetectable levels by 1.5–2.5 years after experimental BIV infection in cattle (Isaacson *et al.*, 1995; Suarez *et al.*, 1995), even though virus was recovered or demonstrated by PCR from PBMC of each BIV-infected animal before and after the loss of p26-specific antibodies. In contrast, immune reactivity to the envelope TM protein of BIV, which appears later in the course of BIV infection, was still detectable at the end of the experiment period (up to 3.5 or 4 years after infection). These results are in accordance with our own data, in which immune reactivity to the BIV TM was detected in cattle whose sera failed to recognize the p26 protein (Abed *et al.*, 1999; Abed and Archambault, 2000). Whether these changes in antibody production reflect differences in the apparently differential expression of the *gag* and *env* gene products *in vivo* late in infection is at present unknown.

The BIV regulatory Tat protein and TAR element in comparison with those of other lentiviruses

Regulatory/accessory genes/proteins are important features that differentiate lentiviruses from other retroviruses. One of the most studied regulatory proteins is the Tat protein, which increases the levels of viral gene expression. Although all lentiviruses code for Tat, the Tat proteins can be classified into two functional groups. The first group of Tat proteins is found in BIV, JDV, HIV-1 and HIV-2, SIV and EIAV. These viruses transactivate their respective LTRs through interactions between Tat, cyclin T1 (cycT1) cellular factor, and the TAR element present at the 5′ end of all viral RNA transcripts (Gunnery *et al.*, 1992; Southgate and Green 1995; Mhashilkar *et al.*, 1997; Willbold *et al.*, 1998; Barboric *et al.*, 2000). Tat exerts its role by enhancing the rates of elongation in these viruses rather than initiating the transcription by using the cellular RNA polymerase II (RNA polII). The second group of Tat proteins is found in MVV, CAEV and FIV, which weakly transactivate their homologous LTRs in a TAR-independent manner (Harmache *et al.*, 1995). The TAR element is absent from the viral transcripts in these viruses and the Tat proteins act through transcription factor binding sites located in the U3 region of the LTR.

BIV Tat is a nuclear and nucleolar phosphoprotein of 14 kDa that is encoded by a multiply spliced mRNA composed of one untranslated leader sequence (exon 1) and two encoding exons (exons 2 and 3) (Carpenter *et al.*, 1992; Liu *et al.*, 1992; Pallansch *et al.*, 1992; Fong *et al.*, 1995, 1997). Exon 2 only codes for a *tat* product of 103 amino acids (Tat103), sufficient to transactivate the BIV LTR (Fong *et al.*, 1997). In addition to Tat103, a Tat protein of 108 amino acids (Tat108) was described (Fong *et al.*, 1997). BIV Tat108 is generated by using different donor and acceptor sites, and comprises the first 98 amino acids of exon 2 and 10 amino acids from exon 3. Similarly, two forms of Tat protein (Tat86 and Tat101) were found in HIV-1 by using alternate splicing (Jeang *et al.*, 1999).

BIV Tat contains the five structural domains common

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to primate lentiviral Tat proteins e.g. acidic amino terminal, cysteine-rich, highly conserved core, basic and carboxy-terminal regions (Fig. 4) (Ruben *et al.*, 1989; Liu *et al.*, 1992; Carpenter *et al.*, 1993; Gonda *et al.*, 1994; Willbold *et al.*, 1994; Fong *et al.*, 1997; Taube *et al.*, 1999). The functional domains of BIV Tat include an activation domain and an RNA-binding domain. The activation domain is composed of the N-terminal, cysteine-rich and central core structural domains that are essential for the interaction of Tat with the cycT1 cell protein (Bieniasz *et al.*, 1998; Bogerd *et al.*, 2000). The RNA-binding domain contains a basic sequence rich in arginine residues (GPRPRGTRGKGRRIRR), which includes the nuclear localization signal (NLS) (Bieniasz *et al.*, 1998; Efthymiadis *et al.*, 1998). Tat protein residues, directly interacting with the RNA, include Arg70, Gly71, Thr72, Arg73, Arg77 and Ile79, whereas the Gly74 and Gly76 residues appear to play a conformational role (Chen and Frankel, 1995; Puglisi *et al.*, 1995; Greenbaum, 1996; Moras and Poterszman, 1996). The BIV Tat protein adopts a β-sheet conformation as opposed to other lentivirus Tat proteins adopting a helical structure (Willbold *et al.*, 1993; Chen and Frankel, 1994, 1995; Puglisi *et al.*, 1995).

Lentivirus gene expression associated with Tat function is dependent on the phosphorylation of cell factors. Initiation of transcription starts in the 5′ LTR by the recruitment of usual cellular transcription factors (Fig. 5).

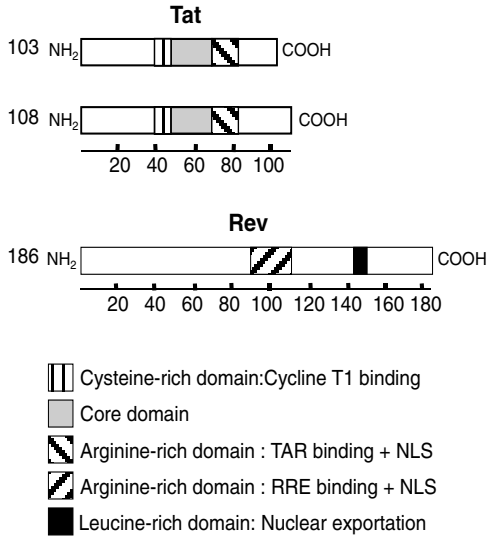

Fig. 4. Schematic representation of the structural domains of the bovine immunodeficiency virus (BIV) Tat and Rev proteins. The Tat proteins (Tat103 and Tat108) are composed of an acidic amino-terminal, a cysteine-rich, a central core, an arginine-rich and a carboxy-terminal domain. The major domains of Rev protein consist of an arginine-rich and a leucine-rich domain, which contains the nuclear export signal. The arginine-rich domains of Tat and Rev contains the nuclear import signal (NLS) and are involved in the binding of RNA to the transactivation-responsive element (TAR) and the Rev-responsive element (RRE), respectively.

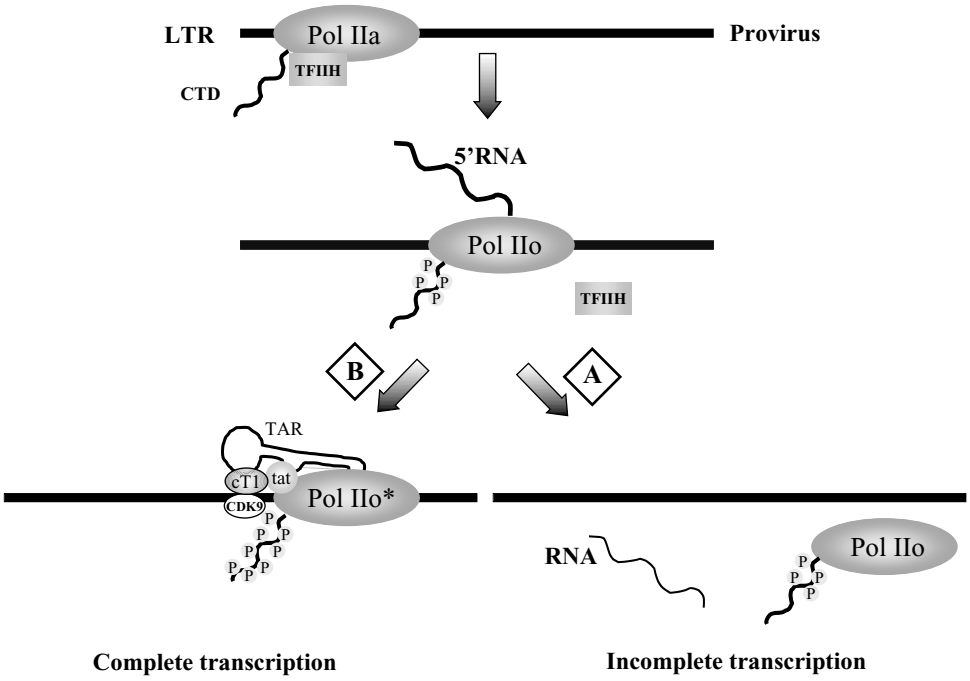

Fig. 5. Model of TAR-dependent Tat transactivation. The pre-initiation complex formed by RNA polIIa and TFIIH binds to the LTR. Viral transcription is initiated when the CDK7 subunit of TFIIH phosphorylates the carboxy-terminal domain (CTD) of RNA polIIa to generate RNA polIIo. In the absence of Tat, the transcription event is not highly processive, resulting in the production of abortive transcripts (A). In the presence of Tat (B), the pTEFb factor (composed of cyclin T1 and CDK9) is recruited by Tat to the TAR RNA element. Once bound to TAR RNA, CDK9 phosphorylates the CTD of RNA polIIo to generate the hyperphosphorylated(*) form of RNA polIIo. This leads to highly processive elongation, resulting in increased production of full-length transcripts. Circles with the letter P indicate phosphorylation events.

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The hypophosphorylated form of cellular RNA polymerase IIa (RNA polIIa) is recruited to the viral promoter by the transcription factor II D (TFIID) in association with the cyclin-dependent kinase 7 (CDK7) which is a subunit of the transcription factor II H (TFIIH) (Garcia-Martinez *et al.*, 1997; Karn, 1999). CDK7 phosphorylates the serine residues present in the carboxy-terminal domain (CTD) of RNA polIIa (Okamoto *et al.*, 1996; Garber *et al.*, 2000). This phosphorylation process allows the RNA polIIo, the phosphorylated form of RNA polII, to initiate transcription at the junction of U3 and R (Ping and Rana, 1999), resulting in the synthesis of the TAR element (Feng and Holland, 1988; Cullen, 1998; Yankulov and Bentley, 1998). It is noteworthy that the transcription complex becomes unstable and inefficient in ensuring complete transcription in absence of Tat, resulting in the accumulation of short RNA strands into the nucleus that ultimately will be degraded by cellular RNases (Greenbaum, 1996).

Tat binds to the newly synthesized TAR element in TAR-dependent transactivation, and then interacts with the positive transcription elongation factor b (pTEFb), directly acting on the RNA polIIo (Fig. 5) (Cujec *et al.*, 1997b; Mancebo *et al.*, 1997; Zhou *et al.*, 1998; Zhang *et al.*, 2000). The pTEFb factor is composed of two molecules, cyclin-dependent kinase 9 (CDK9) and cycT1. CycT1, to which CDK9 is complexed, binds to the cysteine-rich domain of Tat (Chen and Frankel, 1994; Cujec *et* al., 1997a; Fujinaga *et al.*, 1998; Barboric *et al.*, 2000; Bogerd *et al.*, 2000). Then, CDK9 acts on the CTD of RNA polIIo, which becomes hyperphosphorylated (RNA polIIo*) (Herrman and Rice, 1995; Gold *et al.*, 1998; Isel and Karn, 1999; Okamoto *et al.*, 1999; Ping and Rana, 1999). This phosphorylation step consolidates the RNA polIIo* binding on the provirus DNA in order to achieve efficient and complete elongation of viral transcripts.

The TAR element varies in length and structure among lentiviruses. Then, Tat is differentiated from their homologous TAR element (Fig. 6). The TAR RNA of BIV, JDV and HIV-1 forms a stem–bulge–loop hairpin structure composed of 28, 27 and 59 nucleotides, respectively (Colvin and Garcia-Blanco, 1992; Chen and Frankel 1994, 1995; Lustig *et al.*, 1998; Chen *et al.*, 1999b). The TAR RNA is a 25-nucleotide stem–loop structure that lacks the bulge in EIAV (Derse *et al.*, 1991; Hoffman and White, 1995). Although EIAV transactivation was demonstrated to be dependent on the pTEFb factor, EIAV Tat does not harbor a cysteine-rich structural domain, as do other lentiviruses (Dorn *et al.*, 1990; Derse and Newbold, 1993; Albrecht *et al.*, 2000; Sune *et al.*, 2000). Tat interacts in BIV transactivation with nucleotides G11-C25, G14-C23 and C15-G22 located in the stem of TAR, and it directly binds the bulge at U10 (Chen and Frankel, 1994, 1995). Then, a triple-base RNA structure composed of nucleotides U10–A13–U24 is made and is consolidated by hydrogen bonds (Moras and Poterszman, 1996; Lim and Barton, 1997). The central loop in BIV TAR, made of the CAUU residues, is not essential for the binding of BIV Tat as opposed to HIV (Chen and Frankel, 1994, 1995; Puglisi *et al.*, 1995; Barboric *et al.*, 2000).

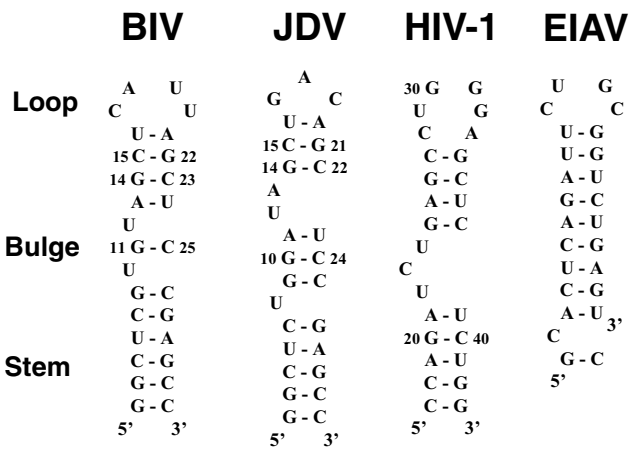

Fig. 6. Schematic representation of various lentivirus LTR TAR structures. The TAR RNA of BIV, JDV and HIV-1 forms a stem–bulge–loop hairpin structure, whereas EIAV TAR RNA forms a stem–loop structure that lacks the bulge. Adapted from Derse *et al.* (1991), Lustig *et al.* (1998) and Chen *et al.* (1999b).

Although the TAR-dependent transactivation mechanism shares similarities among lentiviruses, there are differences in the Tat-pTEFb-TAR recognition event and complex formation that are necessary for efficient virus gene expression. The ability of Tat to recruit pTEFb to TAR determines the host range of Tat function (Bieniasz *et al.*, 1998; Chen *et al.*, 1999a; Kwak *et al.*, 1999; Albrecht *et al.*, 2000). For instance, although murine cycT1 interacts with the HIV-1 Tat activation domain, the HIV-1 Tat transactivation activity is obtained at a very low level in murine cells. However, HIV Tat activity can be rescued in these cells through the exogenous expression of the human cycT1 (Bieniasz *et al.*, 1998; Garber *et al.*, 1998; Kwak *et al.*, 1999). Similarly, the EIAV Tat protein uses equine but not human cycT1 to activate the EIAV LTR (Albrecht *et al.*, 2000; Taube *et al.*, 2000). To explain such discrepancies in lentivirus Tat activity, it was indicated that a single amino acid difference in cycT1 from mammalian species is sufficient to determine distinct RNA binding properties of Tat (Garber *et al.*, 1998). Another explanation refers to the interaction of Tat with cycT1, which appears to increase the affinity and specificity of the binding between Tat and TAR in some lentiviruses. For instance, a preformed complex, Tat–pTEFb, binds to TAR in HIV (Barboric *et al.*, 2000; Bogerd *et al.*, 2000). In contrast, BIV Tat may recognize the BIV TAR with high affinity in the presence or absence of cycT1 (Chen and Frankel, 1994, 1995; Puglisi *et al.*, 1995; Ye *et al.*, 1995; Taube *et al.*, 1999; Barboric

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et al., 2000). Consequently, BIV Tat–TAR interaction takes place in most mammalian cells, including murine, canine, rabbit and human cells, indicating flexibility for BIV Tat to recruit cycT1 (Chen and Frankel, 1994; Barboric *et al.*, 2000; Bogerd *et al.*, 2000; Das *et al.*, 2004).

As mentioned above, the MVV, CAEV and FIV Tat proteins weakly transactivate their homologous LTR in a TAR-independent manner (Harmache *et al.*, 1995). The MVV and CAEV Tat proteins, made of 94 and 86 amino acids respectively, reveal similar structures, with N-terminal acidic and hydrophobic, central leucine-rich and C-terminal cysteine-rich domains (Jackson *et al.*, 1991; Saltarelli *et al.*, 1993; Kalinski *et al.*, 1994). The N-terminal domain of these Tats interacts with the TATA binding protein, whereas the leucine-rich domain interacts with the cellular factors Jun and Fos, which bind to the AP-1 transcription sites located in the U3 region of LTR, thus resulting in efficient viral gene expression (Gdovin and Clements, 1992; Carruth *et al.*, 1996; Morse *et al.*, 1999). Unlike other lentiviruses, the FIV genome does not have a clearly defined *tat* gene. Instead, it carries an ORF, called ORF-A, encoding a Tat-like protein of 79 amino acids. Conflicting data on the ability of ORF-A to transactivate the FIV LTR were previously reported. Indeed, upregulation of the FIV LTR promoter activity mediated by the ORF-A gene product was demonstrated (De Parseval and Elder, 1999; Chatterji *et al.*, 2002). However, a recent study indicated that ORF-A does not affect the viral gene expression *in vitro*, although it is still necessary for virus infectivity (Gemeniano *et al.*, 2003). By this means, the ORF-A-encoded protein would appear to be more similar to the accessory proteins Vpr, Vpu and Nef than to the Tat protein of other lentiviruses. Whatever the role of ORF-A in transactivation may be, it is noteworthy that the FIV LTR U3 region contains recognition sequences for the cellular transcription factors AP-1, AP-4, ATF (the cyclic AMP response element), NF-κB and C/EBP, which are indeed essential for LTR promoter activity. Consequently, the ORF-A gene product would indirectly enhance viral transcription through interactions with these cellular transcription factors (Kawaguchi *et al.*, 1995; Chatterji *et al.*, 2002).

The BIV Rev protein in comparison with that of other lentiviruses

Beside the *tat* gene, lentivirus genomes, as mentioned above, carry another important gene coding for the Rev protein. Rev acts at the post-transcriptional level, whereas the Tat protein regulates viral gene expression at the transcriptional level (Mikaelian *et al.*, 1996; Brice *et al.*, 1999). Rev regulates the expression of viral structural proteins in HIV, SIV and MVV by facilitating the transport of unspliced and singly spliced transcripts from the nucleus to the cytoplasm of infected cells (Felber *et al.*, 1989; Cheng *et al.*, 1990; Tiley *et al.*, 1990). Similar observations were obtained in BIV, where the expression of Rev was shown to positively regulate the appearance of *gag*, *gag-pol* and *env* mRNAs in the cytoplasm of infected cells (Oberste *et al.*, 1993).

BIV Rev is a 23 kDa phosphoprotein localized in the nucleus and nucleolus of infected cells (Oberste *et al.*, 1991, 1993). It is encoded from a multiply spliced mRNA that contains the untranslated leader (exon 1) and two encoding exons (exons 2 and 3) (Oberste *et al.*, 1991, 1993). Exon 2 is in the same reading frame as the Env-encoding region since the first 42 amino acids of Rev are common to those of Env protein (Oberste *et al.*, 1991; Rasmussen *et al.*, 1992). The regulatory activity of Rev is mediated through its binding to the Rev-responsive element (RRE) derived from a sequence of *env* encoding the extracellular domain of TM (Oberste *et al.*, 1993; Gonda *et al.*, 1994). The RRE is located only at the 3′ end of unspliced and singly spliced viral transcripts (Phillips *et al.*, 1992; Oberste *et al.*, 1993; Schoborg *et al.*, 1994; Abelson and Schoborg, 2003). The RRE forms an RNA stem–loop which varies in length among lentiviruses (for instance, 312 bp in BIV and 204 bp in CAEV) in these transcripts (Schoborg and Clements, 1996; Molina *et al.*, 2002).

Two forms of BIV Rev, 159 and 186 amino acids in length, due to alternate splicing, were observed (Oberste *et al.*, 1993). BIV Rev is predicted to be structurally analogous to HIV Rev with the presence of amino-terminal, arginine-rich, multimerization, leucine-rich and carboxy-terminal domains (Fig. 4). Rev adopts a helical structure typical of nucleic acid binding proteins (Dillon *et al.*, 1991; Carpenter *et al.*, 1997; Hope, 1999). The arginine-rich domain contains the NLS and interacts with the RRE. The leucine-rich domain constitutes the Rev nuclear export signal (NES).

The mechanism of nuclear export of viral RNAs mediated by Rev has been studied mainly in HIV. The functional activity of Rev is regulated by both NLS and NES elements which stimulate a continuous Rev shuttle between the nucleus and the cytoplasm (Zapp *et al.*, 1991; Love *et al.*, 1998; Thomas *et al.*, 1998; Jeong *et al.*, 2000). Following translation, the Rev NLS element binds to an importin-β with Ran-GDP (Fig. 7). This binding allows Rev to cross the nuclear pores. Then, the arginine-rich domain of Rev binds in the nucleus to the RRE element included in the incompletely spliced viral RNAs (Cook *et al.*, 1991; Tiley *et al.*, 1991; Ippolito and Steitz, 2000). Several Rev proteins bind the same RRE through a multimerization event, which is indeed necessary for efficient nuclear export of viral RNAs (Tiley *et al.*, 1992; Cullen, 1998; Thomas *et al.*, 1998). Following binding, the NLS is hidden, and only the NES element of Rev is exposed to cellular factors. The exportin CRM-1 (chromosome region maintenance-1) interacts specifically and directly with Ran-GTP and the NES element of Rev to

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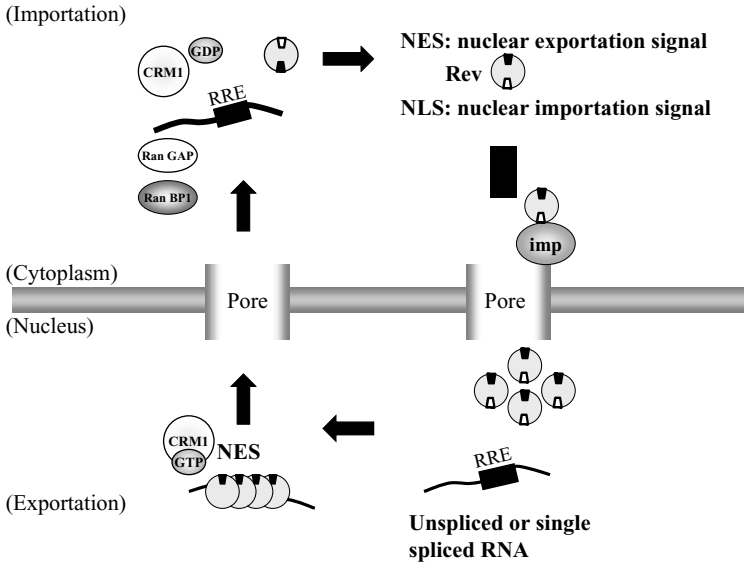

Fig. 7. The Rev export mechanism. Following translation, Rev forms a complex with importin-β, which allows Rev translocation in the nucleus. There, Rev is dissociated from importin-β by the nuclear Ran-GTP. The free Rev protein then binds to the RRE element through its basic domain (which also contains the NLS) to form a multimeric complex by which the NES become accessible for interaction with an exportin (CRM-1). Once exported to the cytoplasm, Rev dissociates from the RRE by the Ran-GTPase, resulting in free Rev that is available for another RNA export cycle.

mediate the transport of the Rev/RRE RNA complexes from the nucleus to the cytoplasm (Fritz and Green, 1996; Nam *et al.*, 2001). There, the CRM-1/Rev/RRE RNA complexes dissociate upon conversion of Ran-GTP into Ran-GDP by an RanGTPase with RanBP1 as a cofactor (Henderson and Percipalle, 1997; Emerman and Malim, 1998), resulting in free Rev that is available for another nuclear export pathway.

The lentivirus accessory proteins

In addition to structural and regulatory proteins, some lentiviruses encode small accessory proteins that are involved in provirus integration, and the assembly or release of new virions (Subbramanian and Cohen, 1994). The negative factor (Nef) protein of human and primate lentiviruses is necessary for the development and maintenance of active infection. Nef plays a particular role in the assembly and release of virus particles by down-regulating expression of the CD4 receptor at the cell surface (Guy *et al.*, 1987; Garcia and Miller, 1991; Anderson *et al.*, 1993; Foster *et al.*, 1994; Sanfridson *et al.*, 1994; Aldrovandi *et al.*, 1998). The BIV genome does not harbor a *nef* sequence, but contains a *tmx* (transmembrane x in reference to its localization in the genome) gene located in a region encompassing the 3′ end of the *env* gene coding for TM and overlapping the 3′ LTR (Garvey *et al.*, 1990). The Tmx protein, with a molecular mass of 19 kDa, was detected in the cytoplasm of infected cells and in the BIV virion (Gonda *et al.*, 1994). Tmx, as Tat and Rev, derives from an early mRNA devoid of the RRE element, and, thus, is Rev-independent for its expression. Although BIV *tmx* and human and primate lentivirus *nef* are similarly located within their respective genomes, the possibility that Tmx exerts Nef functions has yet to be determined. However, the *vif* gene, located downstream from the *pol* gene, was identified in all lentiviruses except EIAV (Rabson *et al.*, 1985; Kan *et al.*, 1986; Lee *et al.*, 1986; Sodroski *et al.*, 1986; Gonda *et al.*, 1994; Kristbjornsdottir *et al.*, 2004). In HIV-1, Vif is a basic protein of 23 kDa which is packaged into virions. Vif, whose expression in BIV has yet to be confirmed, acts late in the lentiviral life cycle and is required for optimal production of new virions (Fisher *et al.*, 1987; Strebel *et al.*, 1987; Borman *et al.*, 1995). In fact, HIV-1 Vif enhances viral infectivity by 10- to 1000-fold (Gabuzda *et al.*, 1992; Von Schwedler *et al.*, 1993; Kao *et al.*, 2003).

Other small proteins are observed in only some lentiviruses. For instance, the HIV-1 and SIV viral protein r (Vpr) and the HIV-2 and SIV viral protein x (Vpx) both promote the transport of the DNA pre-integration complex into the nuclei of non-dividing cells (Lu *et al.*, 1993; Paxton *et al.*, 1993; Lavallee *et al.*, 1994; Pancio *et al.*, 2000). Also, the viral protein u (Vpu) is observed only in HIV-1. Vpu enhances the release of virus particles from infected cells and decreases the formation of cell syncytia due to the degradation of newly synthesized CD4 receptor molecules (Willey *et al.*, 1992; Geleziunas *et al.*, 1994; Chen *et al.*, 1996; Piguet and Trono, 1999). Moreover, two distinct ORFs (*vpw* and *vpy*), unique in

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BIV, are located in the *vif* gene and are predicted to encode proteins W (Vpw) and Y (Vpy), respectively (Gonda *et al.*, 1990). Based on their genomic location, the role of Vpw (with a predicted mass of 7 kDa) and Vpy (with a predicted mass of 10 kDa), whose expression, as for Vif, has yet to be determined, would exert functions similar to those of the Vpr and Vpu of HIV, respectively.

Hybrid regulatory proteins in lentiviruses

Hybrid Tat, Env and Rev proteins, termed Tnv and Tev, are known in HIV-1 (Salfeld *et al.*, 1990; Benko *et al.*, 1990). They were found in African green monkey kidney cells (Cos-7) or human T-lymphoid cells (H9), respectively, infected with a molecular clone of HIV-1. They are produced by the first encoding exon of *tat*, a part of the *env* gene, and the second encoding exon of *rev* through alternate splicing. Although HIV-1 Tnv and Tev have the functional domains of Tat and Rev, they display transactivation activity that is lower than that of the original HIV-1 Tat.

As mentioned above, alternate splicing may result in more than one form of *tat* and *rev* mRNAs. A new BIV Tat protein, called Tat236, was recently found in our laboratory (M.-C. St-Louis, Y. Abed and D. Archambault, submitted for publication). Tat236 derives from a cDNA clone obtained from a new transcript found in BIV-infected cells. We showed that the BIV Tat236 contains most of the first encoding exon of *tat* and a sequence encoded by *rev*. Reporter gene assays indicated that transactivation of BIV LTR by Tat236 is higher than that of the original BIV Tat proteins in several cell lines. Therefore, Tat236 is the first hybrid Tat protein from BIV or any other lentivirus that shows higher transactivation than the original transactivator Tat proteins.

Genetic diversity in lentiviruses and its impact in pathogenesis

Genetically variant retroviruses (associated with the so-called quasispecies) are a result of reverse transcriptase-induced errors, recombinational events, mutations and selective forces that act on the viral population (Boyer *et al.*, 1992; Truyen *et al.*, 1995; Burke, 1997; Mansky, 1998). Genomic variation allows retroviruses to evade the host immune response, alter cell tropism and syncytium induction, acquire drug resistance, and/or inhibit efforts to construct effective vaccines (Fouchier *et al.*, 1992; Milich *et al.*, 1993; Wolfs *et al.*, 1993; Najera *et al.*, 1995). Genetic variability is mostly confined to regions of the genome encoding the SU envelope proteins due to immune pressures (Chirmule and Pahwa, 1996). However, genetic variation in other regions of the genome may occur, including those encoding the regulatory/accessory proteins or nucleic acid sequences involved in viral gene expression or biogenesis.

Variations in the *pol* and *env* genes are associated in HIV with resistance to anti-retroviral drugs and the ability of the virus to evade the immune system, respectively (Rubio *et al.*, 1997; Pieniazek *et al.*, 2000; Vergne *et al.*, 2000; Hsiou *et al.*, 2001). Similarly, genetic variation within the BIV *pol* and *env* sequences was reported (Suarez and Whetstone, 1995, 97; Cooper *et al.*, 1999; Meas *et al.*, 2001). Sequence analysis of the two BIV 106- and BIV 127-developed molecular infectious-cDNA clones shows an overall genomic variability of 1.7%, with 75% of the substitutions occurring in the SU-coding region of the *env* gene (Garvey *et al.*, 1990). DNA sequence analysis of American BIV field isolates, different from the R-29 isolate, indicated substantial genetic variations among different strains (up to 10% divergence in the conserved *pol* gene) (Suarez *et al.*, 1993, 1995). Genetic variation was also shown by in-depth analysis of these isolates as well as size variation by an apparent recombinational event within the second hypervariable region of the SU-coding gene in naturally and experimentally BIV-infected cattle (Suarez and Whetstone, 1995, 1997). The biological significance of this finding was not discussed further. Nevertheless, the overall results of these genomic comparisons, indicating diversity in both product size and sequence, may also suggest a quasispecies phenomenon for BIV. This is consistent with the results of other observations that genomic comparisons of a portion (183 bp) of the *pol* gene from various BIV isolates show non-conservative amino acid changes (Cooper *et al.*, 1999). Moreover, an intra- and inter-individual *env* variation is observed in BIV R-29-infected rabbits (Kalvatchev *et al.*, 2000), indicating further that the potential may exist for the development of BIV *pol* and *env* quasispecies. However, this interpretation needs to be taken with caution since weak viral replication rates in the presence of neutralizing antibodies were accompanied by an absence of antigenic variation in infected cattle (Carpenter *et al.*, 2000).

Similarly to that of the lentivirus structural proteins, variation may occur in regulatory/accessory genes or sequences involved in virus gene expression. Indeed, genetic variation was reported in HIV LTR as well as in the HIV *tat* and *rev* genes in individuals infected with the virus, and the resulting mutated sequences were shown to have an impact on the regulation of virus gene expression or suggested a role in virus virulence (Golub *et al.*, 1990; Martins *et al.*, 1991; Nagashunmugam *et al.*, 1992; Hua *et al.*, 1996; Krebs *et al.*, 1998; Zhang and Dayton, 1998; Peloponese *et al.*, 1999; Hiebenthal-Millow *et al.*, 2003). Moreover, variation in HIV Rev affects the RNA nuclear export and, consequently, the levels of structural protein production (Belshan *et al.*, 1998). Finally, Belshan *et al.* (2001) were

able to correlate, in EIAV-infected ponies, Rev variation and the stage of disease over time. However, the impact of these variations in the virus gene expression or pathogenesis of other lentiviruses remains poorly studied.

Concluding remarks

Substantial progress has been made on BIV in the last 15 years following the demonstration that it was indeed a lentivirus. In this regard, a novel form of BIV Tat protein, termed Tat236, has been found in BIV-infected cells. The significance of Tat236 in the life replication cycle of BIV or its impact in BIV biogenesis is at present unclear. Finally, although BIV induces lifelong persistent infection in cattle, the attribution of clinical disease to BIV is still controversial and there is no overt immunodeficiency state associated with BIV infection. This may be due partly to the fact that most studies have been conducted with the R-29 isolate of BIV. Therefore, there is a need to find new BIV isolates in order to unequivocally establish the pathogenic impact of BIV infection in cattle.

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