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CELLULAR AND EXTRACELLULAR LEVELS OF RETROVIRUS—HOST INTERACTIONS ON THE EXAMPLE OF THE BOVINE LEUKOSE VIRUS. 1. CELL PENETRATION AND INTEGRATION INTO THE HOST GENOME
(review)

V.I. GLAZKO[1, 2], G.Yu. KOSOVSKII[2], T.T. GLAZKO[1, 2], I.M. DONNIK[3]

[1]*Timiryazev Russian State Agrarian University—Moscow Agrarian Academy,* 49, ul. Timiryazevskaya, Moscow, 127550 Russia, e-mail vigvalery@gmail.com (✉ corresponding author), tglazko@rambler.ru;
[2]*Afanas'ev Research Institute of Fur-Bearing Animal Breeding and Rabbit Breeding*, 6, ul. Trudovaya, pos. Rodniki, Ramenskii Region, Moscow Province, 140143 Russia, e-mail gkosovsky@mail.ru;
[3]*Ural State Agrarian University*, 42, ul. Karl Libknecht, Ekaterinburg, 620075 Russia, e-mail ktqrjp7@yandex.ru
ORCID:
Glazko V.I. orcid.org/0000-0002-8566-8717
Kosovskii G.Yu. orcid.org/0000-0003-3808-3086
Glazko T.T. orcid.org/0000-0002-3879-6935
Donnik I.M. orcid.org/0000-0002-8593-7470
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Abstract

Diagnosis and prevention of the retroviral infection spread among farm animals still remain poorly developed primarily due to the fact that a hierarchic cascade of the events which underlie the retrovirus—host interactions involves molecular, intracellular levels, including cell organelles, and extracellular levels associated with the function of cellular immune networks. This paper presents an overview of own and literature data on the interaction of retroviral pathogen on the example of bovine leukemia virus (BLV) with intracellular structures of target cells. Here we consider four stages of the cascade of the events promoting pathogen, including i) introduction into the cell cytoplasm, ii) the synthesis of DNA copies of the viral genome RNA, iii) their transport into the cell nucleus, and iv) provirus DNA introduction into the host genome. The host genes interacting with viral structures are revealed at each stage. Two key processes contribute to genetic variability of retrovirus genome during infectious cycle: two viral RNAs dimerization needed for reverse transcription increases the frequency of recombination between RNA chains (N. Dubois et al., 2018), and provirus cDNA integration into the host genome can lead to activation of mutational and epigenetic events in both the pathogen genome and the host genome (A. Melamed et al., 2018). BLV pathogenesis is divided into two steps, the infectious cycle of mass infection of host target cells and sequential selection of individual infected cell clones. The peculiarity of the integration sites of the host genome is an increased frequency of mobile genetic elements originally closely related to exogenous retroviral infections (N.A. Gillet et al., 2013; T. Miyasaka et al., 2015). The high density of mobile genetic elements is characteristic of the host genomic DNA fragments flanked by inverted repeats of microsatellite AGC and identification sequence of the DNA transposon Helitron. The multiplicity of intracellular targets, whose polymorphism may be the basis of resistance to retroviral infections, allowed us to assume for the first time that the universal critical factor of the infectious process is the integration of proviral DNA into the host genome. It is suggested that the increased sensitivity of cells to productive BLV infection is due to a decrease in the activity of mechanisms involved in the genome protection from transposition activity. In the next communication, we will discuss the relationship between BLV-infected cells and host immune cell networks, which can also have a determining effect on the development of retroviral-induced infection.

Keywords: retrovirus, bovine leukemia virus, infectious cycle, B lymphocytes, bovine leukemia virus receptor, reverse transcriptase, integrase, mobile genetic elements

Retroviruses (*Retroviridae*) are widely spread mammal pathogens regularly causing multi-million damages to the agriculture. A problem in prevention of the infection incidences is low vaccination effectiveness which is poorly researched. Vaccination principle presupposes that in the processes involved in pathogen—host organism interaction there is a point blocking of which by activa-

tion of relevant host's antibody response may interrupt the infection. However, as indicated by a large number of experimental data, retroviral infection is a cascade of events where retrovirus-host interaction occurs at different levels, from intracellular to supracellular. Accordingly, retrovirus resistance may be due to host's intracellular signal, transport, and enzymatic proteins, as well as to characteristic features of host's leukocyte populations determining adaptive and innate immunity.

Present review is aimed to identify key BLV-host interaction stages. In particular, in the first communication we consider events ensuring BLV entering into the target cells and integration of proviral DNA in host genome, and the second communication will scope effects of BLV-proviral DNA integration toward certain components of the host innate and adaptive immunity.

Structure and functioning of Bovine leukemia virus (BLV) genome, stage of induced BLV pathogenesis, and effect on expression of numerous host genes have been described in sufficient detail. However, retrovirus-host interaction mechanisms are complicated and still require further understanding. For instance, from 7 to 22 % cattle have antibodies to BLV, despite the absence of B-cell clones bearing proviral DNA [1, 2]. Up to date, milestone metabolic pathways resulting in lymphatic leukemia under BLV infection, their diversity, and the reasons leading to ineffective immunity against BLV upon vaccination remain unclear. The ideas about various mechanisms determining resistance to BLV are not yet developed. This adversely interfere preventive measures. Besides, lack of such information challenges and impairs the accuracy of diagnostics and indication, as well as prognosis of infected animal danger to others and individual peculiarities of pathogenesis.

Present communication fills up this gap in part. Analysis of the sources of potential resistance during BLV infection at cellular level which we have performed denotes variety of retrovirus-target cell interactions and multiplicity of cellular mechanisms of retroviral infection resistance. Herewith, it appears that events associated with proviral DNA-host genome integration have crucial significance. We believe that reduction of intracellular control over transposition of mobile genetic elements closely related to retroviral infections by their origin play the main role.

Bovine leukemia virus entry into a target cells. BLV (*Deltaretrovirus* genus, *Orthoretrovirinae* subfamily, *Retroviridae* family) is philogenetically closely related to Human T-lymphotropic virus type 1 (HTLV-1) [3]. HTLV-1 infects human CD4+ T-cells, while BLV infects bovine B-cells; both viruses integrate DNA copies of their genomic RNA into the host genome as a proviral DNA [4, 5]. In BLV infected animals, the pathology is initially symptomless, then an increased proliferation of B-cells in some animals occures and, finally, nearly 5 % animals form B-cell lymphomas. BLV causes significant economic damage to dairy and beef cattle farming [6]. Development of effective immunization methods is so far unsuccessful [7]. In addition to the most common 8 serotypes of BLV derived from mutations in gene *g51*, encoding the surface glucoprotein [8], variants of nucleotide substitutions not related to these mutations have been described. According to this, 28 fully sequenced BLV genomes were divided into A, B and C groups with high, average, and low pathogenicity, respectively, as assessed by in vitro virus replication and by in vivo virus load in cows [9].

Proviral DNA integration results in significant changes in both virus and host cell genomes and may be considered as an example of insertional mutagenesis [10-12]. There are two main steps of BLV-induced infection process: massive integration of proviral DNA into B-lymphocyte genone and prolifration of several infected B-clones [13]. Peripheral blood cells with proviral DNA which are detected in cattle infected with BLV are less than 1 % [3]. Following the first infection

steges, proviral DNA massively integrates into actively transcribed regions of the host genome, but, in furtherance, the integrated copies survive only in single cells and only few of them result in tumorogenesis [13]. Infection process is accompanied by massive death of cells with integrated proviral genome [14]. This is due both to a response of the host's immune system to virus antigens and to proviral DNA integration into actively transcribed host DNA regions, which may decrease cell viability, especially when proviral DNA is incorporated into the regulatory or coding sequences of vital structural genes [13].

Generally speaking, natural selection acts against cell populations with actively expressed proviral DNA. Besides, it was found that BLV infected B-cells are divided into two subpopulations, in one of which IgM and BLV RNA are actively expressed, whereas the other one lacks both IgM and viral RNA expressions. It is assumed that it is the second cell population which becomes predecessor of lymphoma, since proviral DNA (plus-strand) is not expressed in tumor cells [15]. The accumulating data show the expression of micro-RNA of BLV proviral DNA, transcription of which occurs in the B-lymphocytes not expressing full-size BLV genome (positive strand), to be the key regulatory factor promoting initiation of B-cell neoplastic transformation [16-18].

Mechanisms to ensure BLV penetration into target cells. BLV is spreading together with liquids containing cell component (blood, milk) and mainly infects CD5+ IgM+ B-lymphocytes [19]. The accumulated experimental data shows that BLV mainly enter target cells indirectly (i.e. not by direct penetration of free viral particles), but through integration of virus-infected and not infected cells [20]. Intercellular transfer of infection is ensured by close interaction of two glycoproteins bound to lipid layer of virion envelope, the surface protein (SU) and transmembrane protein (TM). Both glycoproteins are products of post-translational breakdown of the predecessor encoded by *eny* gene, which have a unique disulphide bond between the cysteic motifs of SU (CXXC) and TM (CX_6CC). Analysis of retrovirus spread events give a scheme of the fusion induced by the interaction between SU receptor-binding domain (RBD) and receptor in the target cells (21, 22) (Fig. 1).

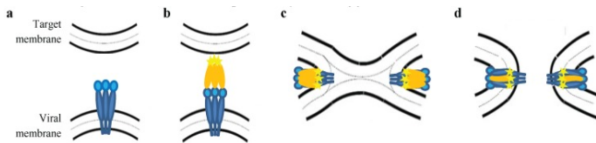

Fig. 1. Bovine leukemia virus (BLV) coat protein membrane fusion (according to the model for murine leukemia virus) [22]. Fusion incompentent state of the envelope complex formed by the receptor-binding (surface protein (SU), gp51 in light blue) and the fusion (transmembrane protein (TM), gp30 in dark blue) subunits (a). After receptor binding, a conformational change exposes the fusion peptide (yellow star) to the target cell membrane (b). Insertion of the fusion peptide into the lipid bilayer mediates formation of a hemifusion diaphragm and blending of viral and cellular lipids (gray dots) (c). Fusion structure after refolding. In this state the fusion peptide and the TM are anchored into the same membrane in an anti-parallel conformation (d) [22].

SU receptor-binding domain (RBD) interacts with specific receptor(s) in target cells. This interaction induces conformational changes initiating TM-directed fusion process. Breaking of disulfide bridge between SU and TM after binding to the receptors provides TM conformation required for membrane fusion. The fusion peptide located at the NH2 end of the TM destabilizes the cell membrane, which allows the virus nucleocapsid to enter the cytoplasm of the host cell [22]. It is assumed (and there is a number of experimental proof) that adaptor-related protein complex-3 (AP-3) participating in protein transport is the main RBD BLV cell receptor [23-25]. This complex participates in formation of transport vesicles to unite the Golgi apparatus and lysosomes and is highly con-

servative among mammal species (88 % identity of amino acid sequences in humans and cattle, and 99 % identity in sheep and goats). There are two main domains in human AP3D1, the adaptine and BLV receptor (Fig. 2). Comparison of amino acid sequences of these domains revealed 15 differences in the second domain (virus receptor) with full absence thereof in the adaptine domain [25].

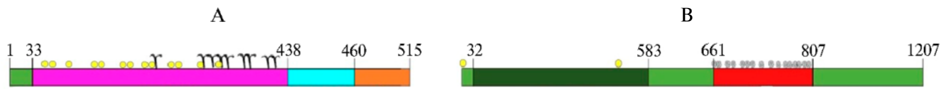

Fig. 2. Bovine leukemia virus coat protein (BLV, *env* gene) (A) **and human adaptive protein complex AP3D1** (B) **[25].** Positions 1-33 — signal peptide (SP), 34-438 — surface protein (SU, gp51), 439-460 — transmembrane protein (TM, gp30), 461-515 —cytoplasmatic region of protein molecule (CR). Zn^{2+} ion binding sites containing cysteine and histidine amino acid residues are shown by yellow circles, N-glycosilation sites (ϒ) are marked (A). Protein AP3D1 (1207 amino acids) contains two domains, the adaptine (32-583) and BLV receptor (661-807). AP3D1 bears two sites of binding Zn^{2+} ions (yellow circles) and 16 conservative sites in BLV receptor domain (grey circles) (B) [25].

Previously we have shown that expression of BLV receptor AP3D1 gene in BLV-infected cows is higher, but does not correlate with an increase in the number of lymphocytes [2]. The research data evidence on expression of this gene in junior B-lymphocytes which reduces with aging [24]. Regardless of the lack of statistically significant correlations between the number of lymphocytes and *ap3d1* gene expression, we have found statistically significant ($p < 0.05$) increase of *ap3d1* expression in animals with BLV proviral DNA as compared to the infection-free individuals. These suggest a relatively increased proportion of junior B-lymphocytes in cell population of BLV-infected animals.

Given wide occurrence of AP3D1 in organs and tissues, its conservatism in the considered mammal types, and pleiotropic mutation effects in gene encoding this central sub-unit of AP-3 complex ensuring transport of membrane proteins to lysosomes [26], one can hardly conceive mechanisms of AP-3 participation in BLV selective penetration exactly into B-lymphocytes. Nevertheless, a number of experimental papers points out to availability of such mechanisms [25]. Complexity of dynamic protein exchange processes between endosomes, plasmatic membrane, and lysosomes allows us to suggests that various ligands, cell elements of cytoskeleton, and other factors which are difficult to control in direct experiments may ensure specific interaction between

Thus, BLV gets into cells of various tissues in different mammalians but can produce full virus progeny only in several of them and only in B-lymphocytes. Accordingly, it is possible to block BLV reproduction at next steps of infection (reversal transcription, integration nto host gene, multiplication of infected cells) [27] that, nevertheless, does not excludes cell resistance to BLV penetration into target cells.

F o r m a t i o n   o f   p r o v i r a l   D N A. Once BLV gets into cell cytoplasm, the viral RNA genome acts as a matrix to synthesize DNA copies (cDNA). Effectiveness of cDNA synthesis significantly depends on successful dimerization of two molecules of virus RNA [28]. At this stage, recombination frequency between two strands of virus RNA is high, and may cause wide spectrum of aberrant proviral genomes.

Interaction between the host's tRNA and long terminal repeats (LTR) of virus genome plays a significant role in initiation of retrotranscription. 5′- and 3′-LTRs are complexes of regulatory motifs required for production of DNA copy of virus RNA and further transcription of proviral DNA. Mutations in LTR, as well as changes in their methylation pattern, may significantly affect the BLV-induced infection (Fig. 3) [29]. 5′- and 3′-LTR regions of virus genome

are initially identical, and differences in their nucleotide sequences (e.g. nucleotide replacements and indels) appear after integration into the host genome. The sequence of LTR retrovirus includes three main functional units, i.e. TG...CA box with TG at 5′-end of 5′-LTR and CA at 3′-end of 3′-LTR; TSR (target site repeat) region of ~ 4-6 bps (short direct repeat flanking 5′- and 3′-ends; this is a "signature" of sites involved in insertion of proviral DNA into the host genome); PBS (primer binding site), a 18 bps sequence complementary to 3′-end of some tRNA located near 3′-end of 5′-LTR (this site is very important since revers transcription starts from binding with tRNA).

Transport of proviral DNA into nucleus of the host cell. Many researches had experimentally shown that replication, formation of pre-integration complex, and further interaction with proteins of nuclear pores during proviral cDNA transfer through the nuclear membrane is due to activity of microtubules of cytoskeleton and microtubule-organizing centre (Fig. 4) [30]. Involvement of cytoskeleton and many host proteins in transport of pre-integration complex to nucleus is characteristic of all retroviruses. Genome of members of *Retroviridae* family including two subfamilies, *Spumaretrovirinae* (*Spumavirus* genus) and *Orthoretrovinae* (*Alpharetrovirus*, *Betaretrovirus*, *Gammaretrovirus*, *Deltaretrovirus*, *Epsilonretrovirus,* and *Lentivirus* genus), consists of two copies of single-stranded RNA. As it was already noted, virion upon entry into a cell is bound by specific receptor surface cell protein, and virion and host membranes are fused (the event occurs either on cell surface or after internalization in endosomes). As a result, virus capside bearing viral genome and reverse transcriptase gets into cytoplasm. In the cytoplasm, reverse transcriptase, after activation by interaction with nucleoside triphosphates, retrotranscribes two-stranded DNA from RNA genome. Resultant cDNA is transported to cell nucleus as part of pre-integration complex (PIC). PIC composition varies in different retroviruses and different cell types. Although, PIC components are poorly studied, it is known that PIC contains proviral DNA, integrase, and capside proteins.

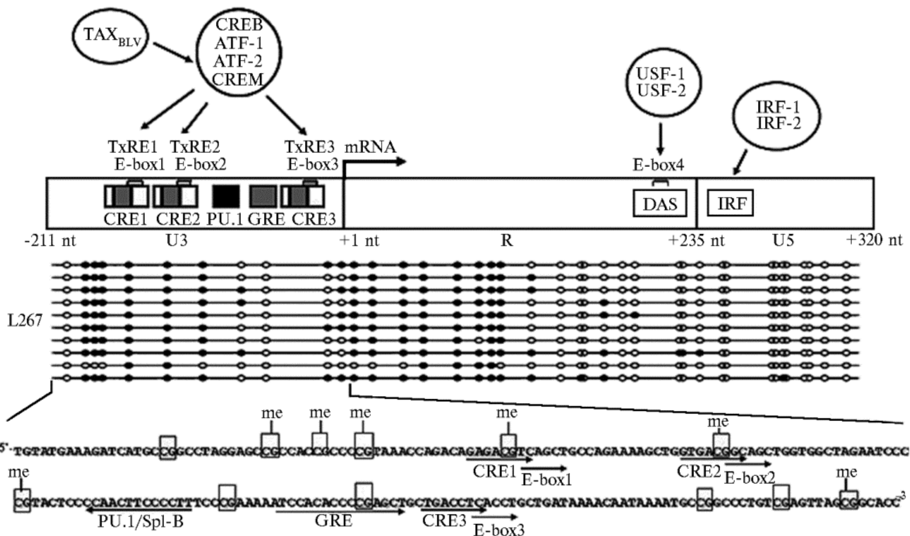

Fig. 3. 5′-LTR of bovine leukemia (BLV) provirus with methylation in CpG (cytosine-phosphate-guanine) motif in sheep cell line L267 at latent BLV infection [29]. mRNA transcription initiation site in region U3—R in 5′-LTR (+1 nucleotide, nt) is marked by arrow. TxRE1, TxRE2, and TxRE3 — three main transcription enhancer sequences of 21 bps each, interacting with host transcription factors CREB, CREM, ATF-1, and ATF-2, which is required for activation of BLV transcription by virus transactivator Tax_{BLV}. Each enhancer contains sequences which are homologous to E-box sequence consensus (E-box1, E-box2 and E-box3) and overlap CRE (CRE1, CRE2, and CRE3 elements regulated by cyclic AMP, i.e. cyclic-AMP responsive element, CRE). U3 region

includes glycocorticoidal regulation element (GRE) (binding of hormone-receptor complex) and site PU.1/Spi-B. USF-1/USF-2 binding site (E-box 4) and interferon-regulating factor binding site (IRF-1/IRF-2) are located in R section of U5 region. Full nucleotide sequence of U3 region with transcription factor binding sites (marked by arrows) and CpG dinucleotides (methylation sites me are highlighted by rectangular) is provided [29].

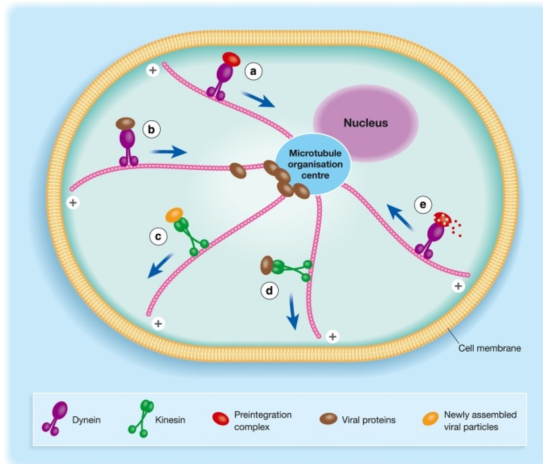

Fig. 4. Motor proteins of microtubules involved in replication of retroviruses [30]. Preintegration retrovirus complexes use dynein for transport along microtubules (a). Newly synthesized retrovirus proteins (Gag and Env) with dynein move to perinuclear zone for virion formation (b). Formed virus particles are further moved by kinesin to cell membrane to exit the cell (c). Viruses assembling at plasma membrane use kinesin for proteins and genome RNA transport (d). Dynein activity is also required to remove virus envelope (E). MOC stands for microtubule-organizing centre. Positive end of microtubules is marked (+), arrows show direction of macromolecule movement through microtubules [30].

Integrated proviral DNA is transcribed by the host's RNA-polymerase II, and virus mRNA is delivered to cytoplasm. Afterwards, they are translated into virus proteins Gag, Pol, and Env (and into auxiliary proteins, if virus genome possesses their genetic determinants), which are later moved to plasma membrane by vesicules, cytoskeleton or otherwise. Following the assembly, immature virus particles bud off the cell membrane. Virion maturing is initiated by viral protease which mediates cleavage of Gag and Gag-Pol proteins. In each described step of this strictly regulated process many host proteins are involved, including the cell cytoskeleton elements. Mutation of genes encoding such proteins may significantly influence the proviral DNA infection success.

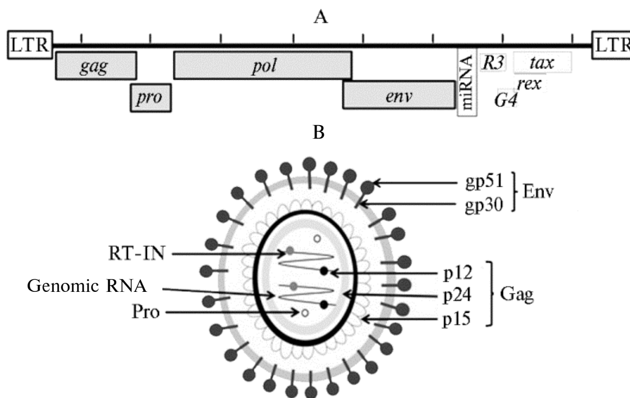

Fig. 5. Genome (A) **and virion structure** (B) **of bovine leukemia virus (BLV) [31].** Structural genes and enzyme genes *env*, *gag*, *pro* and *pol*; regulatory genes *tax* and *rex*; auxiliary genes *R3* and *G4*; microRNA (miRNA) (A). Structural proteins and enzymes: extracellular and transmembrane glycoproteins gp51 and gp30 (Env), Gag proteins — p12 (nucleocapside), p24 (capside) and p15 (matrix), reverse transcripase and integrase (RT-IN) encoded by *pol* gene, protease (Pro) (B).

Structure of BLV genome has been described in detail quite long time ago (Fig. 5) [31]. BLV genome consists of 8714 nucleotides, includes main genes encoding structural proteins and enzymes (mainly, *gag*, *pro*, *pol,* and *env*), and pX region flanked by two identical LTRs. Gene *gag* is translated to the predecessor protein Pr45 which is processed into three mature proteins, i.e. matrix protein P15, which binds virus genome RNA and interacts with lipid bilayer of virus membrane, capside protein P24 (the blood level of antibodies to this protein is high in BLV-infected animals) and nucleocapside protein P12 which is necessary to pack genome RNA (see Fig. 5). Gene *env* encodes mature extracellular pro-

tein gp51 and transmembrane protein gp30. Region pX located between *env* and 3′-LTR encodes regulatory Tax and Rex proteins, as well as auxiliary R3 and G4 proteins (see Fig. 5). Regulatory proteins are involved in control of transcription and export of virus RNA to cytoplasm. In particular, G4 significantly increases virus production [32]. Region encoding five is located between *env* and pX. These microRNAs are transcribed by RNA-polymerase III. Rex and Gag proteins participate in transport of proviral cDNA into cell nucleus. Note, Gag is also bound with centrosome proteins of spindle apparatus, which may lead to multicentric mitosis [33, 34].

Many host proteins, which are partially studied for HIV-1 retrovirus, participate in virion uncoating, transport and cDNA delivery into cell nucleus (Fig. 6) [35].

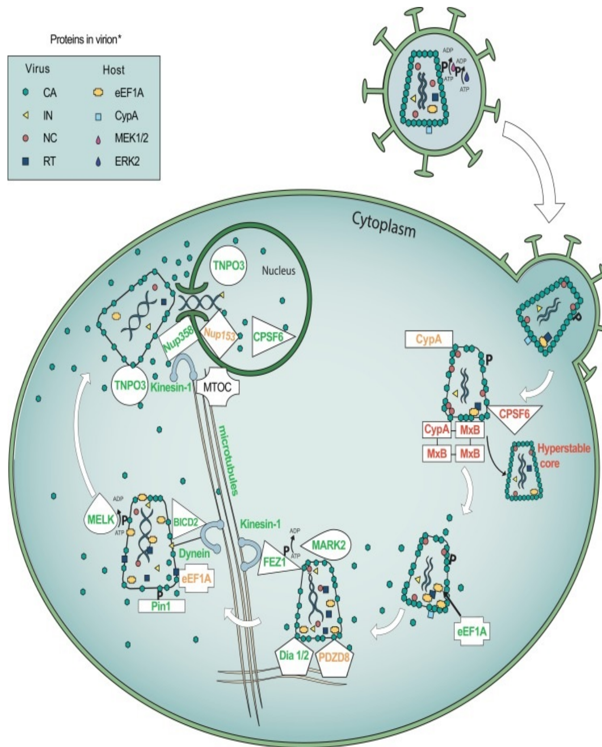

Fig. 6. Factors of host cells involved in uncoating of HIV-1 protein envelope [35]. CA — capside; CPSF6 — specific cutting and polyadenylation factor 6; CypA — cyclophyline A; BICD2 — bicaudal D2; Dia1/2 — formin 1 and formin 2, bound to diaphane; eEF1A — eucariotic elongation factor 1A; ERK2 — kinase 2 regulated by extracellular signals; FEZ1 — fasciculation and elongation factor zeta 1; IN — integrase; KIF5B — kinesin 1 (heavy strand); NC — nucleocapside; NPC — nuclear pore complex; Nup — nucleoporine; MARK2 — kinase 2, regulates affinity of microtubules; MEK1/2 — mitogen-activating protein 1 and protein 2; MELK — maternal embryonic leucine binding kinase; MTOC — microtubule-organizing centres; MxB — Myxovirus B resistance protein; PDZD8 — PDZ domain containing protein 8 involved in interaction between the endoplasmic reticulum and mitochondria; PIC — preintegration complex; RT — reverse transcriptase; RTC — reverse transcription complex; TNPO3 — transportin 3.

Factors of host cell regulate the infection activity of HIV-1. HIV-1 virion contains host proteins CypA, ERK2, and eEF1A regulating fusion with host cell membrane and virion exit to its cytoplasm. PDZD8 and CypA bind CA to stabilize virion, which is required for development of infection; however the infection process is blocked if CypA is bound with MxB forming the complex in which MxB oligomers cause hyperstabilization of virion. Cellular eEF1A interacts with virus RT and activates it. Dia1 and Dia2 bind CA—NC complexes which facilitates their removal (possibly due to local stabilization of microtubules). Dynein interacts with virion through the adapter protein BICD2 or by direct interaction with IN; kinesin 1 interacts with virion through adapter FEZ1 (phosphorylated MARK2). This is important for transfer of replication complexes through cytoplasm to MTOC at core periphery. Pin1 binds CA. Phosphorylated MEK1/2, activated by ERK2 at virion maturing, facilitates its uncoating. MELK also phosphorylates CA, which accelerates this process. Virus RT binds eEF1A for stabilization of RTC. Nuclear pore proteins Nup358 and Nup153 promote importation of PIC, Nup358 moves to cytoplasm with the use of KIF5B, and Nup153 prolongs virion-PIC association in the nucleus. TNPO3 retains CPSF6 in the nucleus to prevent CPSF6-dependent virion hyperstabilization; TNPO3 also facilitates separation of CA from replication complexes in the nucleus. Proteins colored in green ensure optimal virion uncoating kinetics, proteins colored in orange delay uncoating, proteins colored in red cause virion hyperstabilization that suppresses infection. The figure shows only virus proteins and host proteins essential for uncoating and transport of virion [35].

The next crucial step of infection process relates to interaction of pre-integration complex with chromatine. In murine leukemia virus (MOVE) retrotransposon, this interaction is mediated by the product of GAG protein cutting by p12 protein [36]. It is assumed that interaction of virus integrase with cellular serine-treonine phosphatase 2A (PP2A) makes certain contribution to integration of proviral DNA into the host genome [37].

BLV proviral DNA is preferably inserted into the host genome regions rich in CpG (which is typical for promoter regions and protein encoding genes) close to tRNA genes and pseudogenes. tRNA genes (unlike genes encoding proteins and transcribed by RNA polymerase II to mRNA) are constitutively transcribed by RNA polymerase III (Pol III). tRNA pseudogenes lose their ability to produce functional tRNA, but their transcription is still associated with Pol III activity. No predominant integration of BLV proviral DNA in regions with dispersed repeats LINE BovB, SINE BOV-A2, SINE ART2A, and LTR ERV was found [13]. Preferred retrovirus integration occurs into host genome regions with high frequency of palindrome structures [38, 39)], in sites of transcription initiation [41] or in regions having other structural and functional peculiarities, in particular, propensity for interaction with virus integrase [42-44]. Similarity of BLV and HTLV-1 retroviruses is approved by comparison of the parameters of integration sites; for instance, both proviruses are located in actively transcribed regions close to tRNA genes and pseudogenes [13]. According to another research paper based on analysis of 264 sites of BLV integration into bovine genomes, it was concluded that more often such sites are rich in AT-nucleotides and long interspersed nuclear elements (LINE) [45]. Previously, we have shown in own research that density of mobile genetic elements and their recombination products increases in BLV-infected individuals in sequences flanked by AGC microsatellite inverted repeat, as well as in the Helitron transposon identification sequence [46, 47].

Own experimental data allows us to assume that general decrease in activity of protective mechanisms preventing transposition of mobile genetic elements is a factor of propensity for integration of proviral DNA into nuclear genome. It should be noted that preferred location of BLV proviral DNA in the regions of tDNA genes and pseudogenes supports our assumption since multiplication and spreading of pseudogenes is due to transposition of mobile genetic elements, and tRNA pseudogenes are predecessors of a large group of non-autonomous mobile genetic elements of SINE (Short Interspersed Nuclear Element). It is notable that length of found fragments of genome DNA flanked by the inverted repeats of AGC microsatellites, as well as the identification Helitron sequence, varies from 300 to 1500 bps in size and is predisposed to formation of short loops due to flange complementarity.

Transcription of proviral DNA. Transcription of proviral DNA involves i) many factors of cell origins controlled by genes located near the integration region of proviral DNA which interacts with host cell chromatine during integration, as well as ii) regulatory factors encoded by retrovirus genome. Among them, Tax is a key factor [48]. U3 region in 5′-LTR (see Fig. 3) contains canonic promoter of CAT-box (CCAACT in coordinates –97 to –92) and TATA-boxes (GATAAAT between –44 and –38 positions). TxRE is an anchoring motifs containing the elements, functioning of which is regulated by cyclic AMP (cyclic-AMP responsive element, CRE). 21-nucleotide motif also serves as a target to bind Tax protein, a key proviral DNA transcription activator enhancing association between CREB and DNA. In fact, internal CRE-similar motifs (GACGTCA, TGACG, TGAC, and TCA) are close to consensus TGACGTCA. E-box motif (5′-CACGTG-3′) located in front of the site of transcription initiation connects basic transcription factors USF1 and USF2. Many genes of such factors are lo-

cated in motif R. Interferon-regulating factor (IRF-1 and IRF-2) binding sites stimulating the basal expressions without Tax are located in U5.

At post-transcription level, BLV expression is regulated by virus protein Rex which interacts with RNA sequence in 3′-LTR located between AATAAA signal and polyadenilation site. This site with increased frequency of a stable hairpin is bound with two signals of transcription termination. Export of viral transcripts from the nucleus to cytoplasm requires Rex association.

As it was already noted, tumor cells lack Pol II mediated transcription of proviral DNA, while Pol III mediated transcription of DNA regions encoding five BLV microRNAs occurs. It was shown that microRNAs are involved in regulation of many key cellular processes in normal state and during various diseases, including oncopathologies [49]. Transcription of microRNAs in BLV-infected tumor cells causes changes in expression of a set of genes related to signal functions, immune system, and oncogenesis [16, 17, 50]. It was also found that second strand of BLV proviral DNA is also actively transcribed in the infected B-lymphocytes and tumor cells, provided that microRNA participates in destruction of antisense transcript [51]. It is assumed that the balance between transcription of positive and negative strands of proviral DNA in BLV and HTLV-1 defines latent or reactivated viral forms [52].

Therefore, the available data provide the evidence of diverse interactions between the BLV retrovirus genome and host cells. Herewith, key events occur when virion invades a cell and interacts with transport host systems which provide the exchanges between plasma cell membrane and intracellular organelles. The next step is reverse transcription of virus RNA, resulting in cDNA which are further delivered into the nucleus to multiply integrate into the host genome as proviral cDNA (infection cycle). The proviral cDNA is intracellularly transcribed and reproduced, and, finally, predominant replication of several infected cell clones occurs. Given such diversity, infection process may be stopped at any of these steps due to polymorphism of genes (either in pathogen, or in host) which products are involved in a cascade of such events. Infection itself increases frequency of mutagenic events (both during reverse transcription of two strands of virus RNA, and during proviral DNA integration). As a result, nearly 1 % of the infected target cells initiate actively proliferating clones. Since detection of antibodies induced by viral envelope proteins disallows reliable identification of the infected individuals and highly efficient vaccination, it is apparent that events related to integration of proviral DNA with host gene are very important for the infection cycle. The available experimental data allows us to presuppose that key factor at this step is a decrease in intracellular control of transposition of the host's mobile genetic elements closely related, by their origin, to retrovirus. Such assumption is supported by data on i) preferred insertion of BLV proviral DNA into regions with high density of tRNA pseudogenes, LINE, and on ii) higher frequency of mobile genetic elements in genome regions flanked by the inverted repeats of several microsatellites and identification Helitron motifs in BLV-infected cows as compared to infection-free animals. In the next communication we will focus on interactions of BLV-infected cell and cellular networks of the host's immune system which may have controlling influence on proceeding of the retrovirus-induced infection.

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