[Frontiers in Bioscience 14, 4138-4148, January 1, 2009]

Protein-protein interactions and gene expression regulation in HTLV-1 infected cells

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1. ABSTRACT

Human T-cell leukemia virus type 1 (HTLV-1) propagates in CD4 or CD8 T cells and, after extended latency periods of 30–50 years, causes a rapidly fatal leukemia called adult T-cell leukemia/lymphoma (ATL). Infection with HTLV-1 is also associated with a degenerative neuromuscular disease referred to as tropical spastic paraparesis or HTLV-1-associated myelopathy. HTLV genome, in addition to the structural proteins and retroviral enzymes, codes for a region at its 3' end originally designated pX. The products of this region (Tax, Rex, p12I, p13II, p30II and HBZ) play important roles in deregulation of cellular functions by either directly disrupting cellular factors or altering transcription of viral and cellular genes. Here, we will review current knowledge of protein-protein interactions that regulate transcriptional functions of proteins encoded by the pX region.

2. INTRODUCTION

Viruses have developed a variety of strategies to modulate the host cells transcriptional apparatus with the aim of optimizing viral mRNA replication and alteration of cellular genes expression. Retroviruses comprise a distinct group of enveloped RNA viruses that replicate by reverse transcribing their RNA genomes to form a DNA copy that integrates into the host cell genome. The integrated proviral DNA is then transcribed by RNA polymerase II (Pol II) to produce mRNAs that are translated into viral proteins and packaged into assembling core particles in the cytoplasm or at the plasma membrane. Retroviruses are obligate parasites with small genomes and, thus, are dependent on host factors for their replication. Among retroviruses, deltaretroviruses are complex viruses that assemble at the plasma membrane and contain a central spherical inner core (C-type morphology). The most famous member of this group is the

Human T-cell leukemia virus type-1 (HTLV-1), the first pathogenic retrovirus discovered in humans 28 years ago (1). HTLV-1 is the causative agent of two major diseases: a rapidly fatal leukemia designated adult T-cell leukemia (ATL) (2) and a neurological degenerative disease known as tropical spastic paraparesis (TSP) or HTLV-1-associated myelopathy (HAM) (3).

The HTLV-1 genome encodes the structural proteins necessary to form the viral core particle (Gag and Env) and the enzymatic retroviral proteins, (reverse transcriptase, integrase and protease). In addition, the HTLV-1 genome contains a cluster of at least five open reading frames (ORFs) within the pX region that are generated by alternative splicing. The *tax* and *rex* genes are the most extensively studied and encode a phosphoprotein of 40 kD, and a protein of 27 or 21 kD proteins, respectively. The other pX genes encode p12I, p27I, p13II, and p30II (4). A novel ORF has been recently identified in the complementary strand of the pX region and encode the basic leucine-zipper factor HBZ (5). Most of these proteins are implicated in the regulation of viral and cellular genes expression. Here, we will review transcriptional and/or post-transcriptional regulation by HTLV-1 proteins.

3. REGULATION OF HTLV-1 GENE EXPRESSION

3.1. Interaction of the HTLV-1 LTR with transcription factors

The HTLV-1 long terminal repeat (LTR) is divided into three regions: U3, R and U5. The U3 region contains elements critical for viral gene expression: the polyadenylation signal, TATA box and transcription factors binding sites. The 21-bp repeats, termed Tax responsive element 1 (TRE1), are the first transcription factors-binding sites identified in the HTLV-1 LTR. Cellular proteins such as CREB and other bZIP family members have been shown to bind TRE1 via a TGACG (T/A) (C/G) (T/A) motif that is an imperfect homologue of the cAMP-responsive element (CRE; TGACGTCA) (6-7). The major protein complexes formed on the TRE1 are CRE-dependent except the Sp-1 transcription factor, which binds to the proximal TRE1 (8). A serum response factor (SRF) binding site (CArG box) and a ternary complex factor (TCF) binding site CCGGAA) are located within a different region of U3 known as Tax responsive element 2 (TRE2) (9) and they recruit several transcription factors including Sp-1, TIF-1, c-Myb, Elk-1 and SRF (10) (Figure 1A).

These cellular proteins control basal transcription regulation of the HTLV-1 LTR promoter and they recruit Tax to fully activate transcription. Tax does not bind DNA directly but rather acts via protein-protein interactions with cellular transcription factors bound to the viral LTR promoter. The cooperative binding of Tax with several of these transcription factors to the HTLV-1 promoter may position Tax to interact and influence the basal transcription components such as TBP, CBP, TFIIA, TFIID and RNA polymerase II (11-15).

3.2. Interaction of Tax with transcriptional activators

Several bZIP proteins including CREB (16-18), ATF-2 (18), ATF-4 (19-20), and c-Jun (21) interact with Tax and can redundantly serve to mediate Tax-activation of the LTR. However, a clear preference for CREB has been shown (22). Both CREB and Tax associate with the viral 21-base-pair repeat as dimers (23). The effect of Tax on CREB-DNA binding can be explained by a two step mechanism where Tax changes the apparent equilibrium constants for both a CREB-CREB dimerization and a (CREB)2-DNA binding step (24) (Figure 1B). The interaction between Tax and CREB results in increased binding of the Tax/CREB complexes to the HTLV-1 21-bp repeats. Compared with CREB alone, Tax/CREB exhibits greatly increased DNA recognition specificity and preferentially assembles on the 21-bp repeats. Phosphorylation of CREB at serine 133 plays an important role in the recruitment of the large cellular coactivator CREB-binding protein (CBP)/p300 to the HTLV-1 promoter (12, 25). Indeed, Tax and the phosphorylated kinase inducible domain (pKID) of CREB bind distinct surfaces of a compact hydrophobic core, termed KIX domain, of CBP/p300 and stabilize the CBP/p300 cofactor on the HTLV-1 promoter (26-27). The KIX domain of CBP/p300 contributes to Tax transactivation by targeting the acetyltransferase activity of the coactivator to the Tax-CREB (Tax/CREB) complex (28). In addition to CBP/p300, the p300/CREB binding protein (CBP)-associated factor (PCAF) is also involved in transcriptional activation by Tax. PCAF interacts directly with Tax, independently of p300/CBP. PCAF is then recruited to the TREs and cooperates with Tax to activate HTLV-1 transcription. In contrast of the p300 stimulation, the PCAF coactivator activity on Tax transactivation is independent of its HAT activity on the viral long terminal repeat (29) (Figure 1B).

3.3. Interaction of Tax with transcriptional repressors

Mammalian cells have evolved a variety of mechanisms to impede retroviruses, which are pathogenic or mutagenic to their hosts. Viruses potentially exploit some of those mechanisms as a strategy for persistence in their host cells. HTLV-1 LTR CRE sites can potentially bind to a variety of bZIP proteins resulting in diverse transcriptional regulatory activities. It has been shown that ATFx (also known as ATF5), the sole member of the CREB/ATF family of bZIP factors that exhibits an anti apoptotic activity (30), inhibits basal and Tax-dependent transcriptional activation by bridging interaction between Tax and the TRE1 (31). The CCAAT/enhancer binding protein beta (C/EBPbeta), another bZIP protein, is able to form stable heterodimers with CREB and repress Tax transactivation function by competing with the ability of Tax to bind homo- or heterodimers of CREB/ATF family members (32).

Histone deacetylases (HDACs) constitute a different group of repressors that could play an important role in HTLV-1 silencing *in vivo*. Histones form the backbone of chromatin. HAT enzymes acetylate lysine residues on histones, neutralize their positive charges and diminish their ability to bind negatively charged DNA. This open chromatin configuration provides accessibility to the general transcription machinery and regulatory factors. Thus, HATs enzymes facilitate transcriptional activation of several genes *in vivo*. On the contrary, HDACs remove

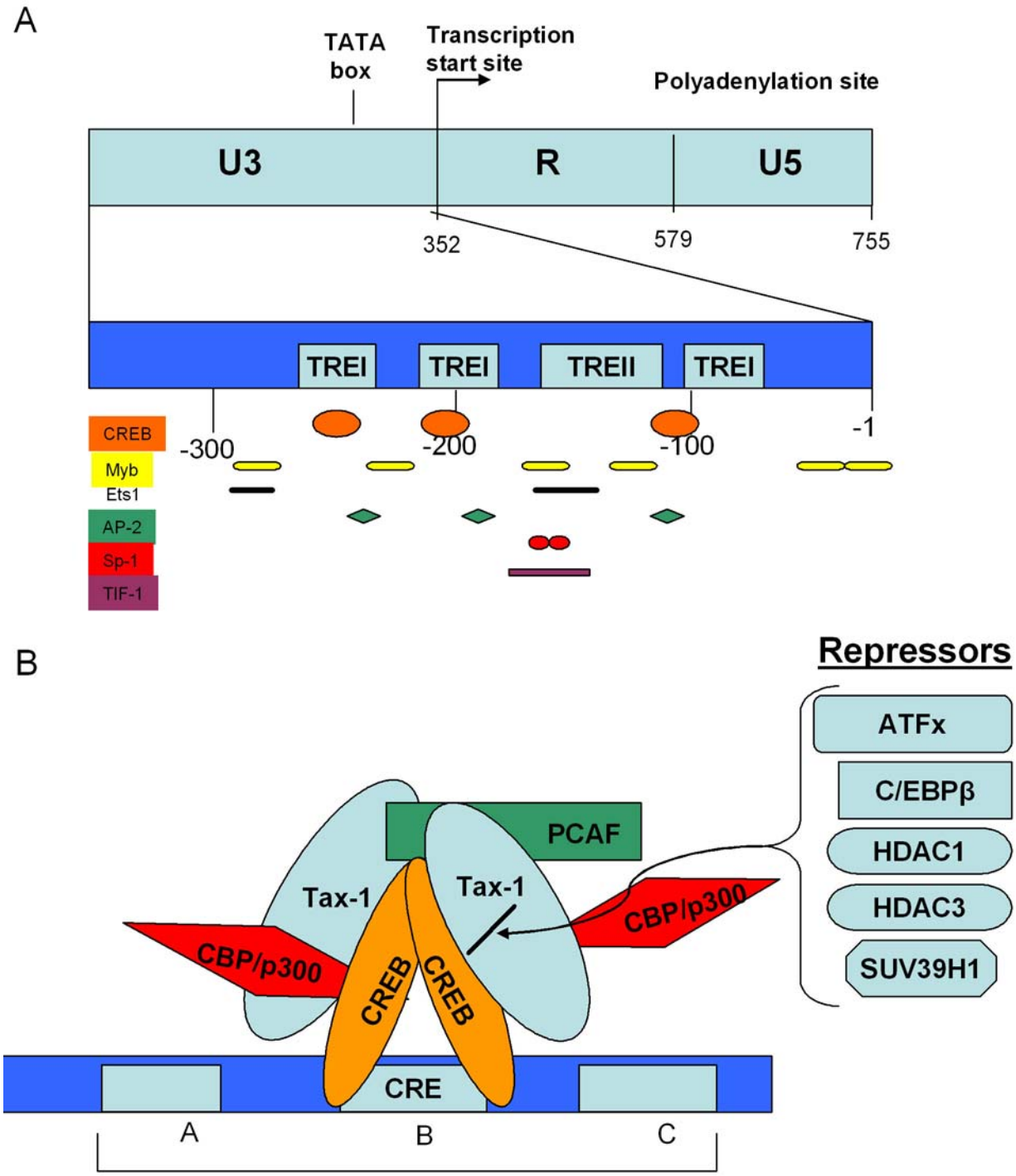

Figure 1. A. Schematic representation of the HTLV-1 LTR. Binding sites of several proteins, such as CREB, Myb1, Ets1, AP-2, Sp-1, TIF-1 are indicated. The three CREB/ATF binding sites (TREI) and the SRF/TCF binding site (TREII) are also indicated. B. Schematic illustration of the DNA elements and regulatory factors involved in Tax-induced transcriptional activation of HTLV-1 LTR. Tax interacts with CREB/ATF proteins, strengthens their dimerization and their LTR binding to induce HTLV-1 expression. Moreover, Tax recruits co-activators (such as CBP/p300 and PCAF) and repressors (such as ATFx, C/EBPβ, HDAC1, HDAC3, and SUV39H1).

acetyl groups from histones, allowing compacted chromatin to reform. HATs and HDACs are not just specific for histones. Many regulatory factors of the cell cycle, DNA repair, recombination and replication, signaling molecules

such as kinases and phosphatases, viral proteins such as HIV Tat are also regulated by HATs/HDACs interplay (33). In the context of the regulation of HTLV-1 genes expression, it has been demonstrated that Tax interacts with class I histone deacetylases HDAC-1 and -3 (34-35). Interaction of Tax with HDACs negatively regulates Tax transactivation function. This repression can be relieved by treatment with Trichostatin A, an HDAC inhibitor, or by overexpression of the transcriptional activator CBP. HDACs are likely to compete with p300/CBP and PCAF in binding with Tax and regulating the transcriptional activation of the HTLV-1 LTR promoter. Tax also interacts with SUV39H1, an histone methyltransferase that methylates histone H3 at lysine 9 (H3K9). SUV39H1 represses Tax transactivation of the LTR in manner dependent on the methyltransferase activity of SUV39H1 (36) (Figure 1B).

Inhibition of Tax transactivation activity also occurs by indirect mechanisms. For instance, the homeodomain protein MSX2, a general negative regulator of gene expression, known to interact with components of the basal transcription machinery such as TFIIF (RAP74 and RAP30) (37) is recruited by Tax and inhibits HTLV-1 LTR activation (38). We have also shown that HTLV-1 Tax can be sequestered out of the nucleus by some cellular partners such as tristetraprolin (TTP) and G proteins beta subunits (39-40).

3.4. Contribution of Rex, p30 and HBZ in regulating HTLV-1 expression

The Rex protein of HTLV-1 has been shown to participate in the production of viral particles. Rex binds to the cis-acting sequences (Rex response element) present at the 3' end of viral mRNAs (41) and selectively export the unspliced *gag/pol* and *env* viral mRNA from nucleus to the cytoplasm in a CRM1-dependent manner. As a result, Rex increases the expression of structural and enzymatic proteins and the formation of viral particles (41). In contrast with Rex, HTLV-I p30II is a nuclear protein that binds to the doubly spliced mRNA encoding Tax and Rex proteins, and retains them in the nucleus. Overexpression of p30 blocks the translocation of *tax/rex* mRNA from the nucleus to the cytoplasm, resulting in the inhibition of viral gene expression and promoting viral latency and persistence *in vivo* (42-43).

The more recently discovered HBZ protein encoded by the complementary strand of the HTLV proviral genome possesses a putative bZIP domain and is able to dimerize with cellular bZIP proteins including CREB-2, c-Jun and JunB. Its interaction with CREB-2 and AP-1 transcription factors suppresses HTLV-1 basal transcription and Tax-transactivation activity (5, 44).

In summary, HTLV-1 has evolved a complex regulatory mechanism of antagonizing viral transcriptional (Tax versus HBZ) and post-transcriptional (Rex versus p30) regulators to allow a control of viral gene expression. The interplay between these viral and cellular regulatory proteins contributes to multiple steps of the leukemogenesis process.

4. REGULATION OF CELLULAR GENE EXPRESSION IN HTLV-1 INFECTED CELLS

The effects of Tax on cellular genes expression have an important impact on HTLV-1 induced transformation. In fact, Tax modulated genes are involved in crucial cellular mechanisms such as apoptosis and proliferation, cell cycle and division, cell migration or immune response and inflammation. Tax transactivates these cellular promoters by interacting with transcription factors such as CREB/ATF, NF-κB, SRF and AP-1.

4.1. The CREB/ATF pathway

In addition to the regulation of viral genes expression, the CREB/ATF pathway is also used by HTLV-1 to deregulate cellular genes expression. For example, the development of ATL has been associated with T lymphocytes containing abnormal chromosomal content, which develops due to aberrant mitotic divisions. Several studies have demonstrated the crucial role of Tax in the development of aneuploidy in infected cells. Tax acts through protein – protein interactions with cell cycle mediators including MAD1/MAD2 (45), Chk1 (46), the anaphase promoting complex (APC)- APC-Cdc20 (47) or cyclin D-cdk and p110Rb (48). Alteration of cellular genes transcription is an additional mechanism used by HTLV-1 to deregulate cell division. Gene expression profiling and bioinformatics promoter analysis identified 95 genes containing CREB binding sites and over-expressed in Tax-expressing cells; 11 of these genes are involved in G2/M phase regulation, in particular kinetochore regulation including Sgt1 and p97/Vcp (49). Another example is the phosphatidylinositol-3-kinase (PI3K) and AKT (protein kinase B) signaling pathways that plays an important role in regulating cell cycle progression and cell survival. Tax activates PI3K/Akt through activating the CREB signaling pathway. Activation of PI3K/Akt pathway leads to β-catenin over-expression in Tax-positive HTLV-1 infected cells. β-catenin has been implicated in the malignant transformation of cells and probably plays an important role in T lymphocytes transformation by HTLV-1 (50).

4.2. NF-κB pathway activation

In mammalian cells, there are five NF-κB family members, RelA (p65), RelB, c-Rel, p50/p105 (NF-κB1) and p52/p100 (NF-κB2), organized in different homo and heterodimer NF-κB complexes. All NF-κB family members share an approximately 300-amino acids N-terminal Rel-homology domain (RHD), which mediates DNA binding, dimerization and nuclear localization. In most cells types, NF-κB complexes are retained in the cytoplasm by a family of inhibitory proteins known as inhibitors of NF-κB (IκBs). There are two principal IκBs, IκBα and IκBβ, which function in part by masking a conserved nuclear localization sequence (NLS) of the RHD. The classical NF-κB pathway is induced in response to various stimuli, including the pro-inflammatory cytokines tumour necrosis factor-alpha (TNFα) and interleukin-1 (IL-1), engagement of the T-cell receptor (TCR) or exposure to viral and bacterial products. Following induction by various stimuli, the IκBs are phosphorylated and degraded by the proteasome. Thus activated, NF-κB translocates to the

nucleus, where it stimulates transcription of genes containing the κB consensus binding site 5-GGGRNNYYCC-3. The IκB kinase (IKK) complex is responsible for IκB phosphorylation. It consists of three core subunits, the catalytic subunits IKKα and IKKβ and several copies of a regulatory subunit called the NF-κB essential modifier (NEMO, also known as IKKγ) (51). In normal T cell, NF-κB activation occurs transiently in response to immune stimuli required for antigen-stimulated T-cell proliferation and survival. T-cell transformation by HTLV-1 involves deregulation of cellular transcription factors, including members of the NF-κB family.

HTLV-1 Tax acts at different levels to activate the NF-κB pathway. (1) IKKγ/NEMO recruits Tax to the IKK catalytic subunits IKKα and IKKβ resulting in their activation (52-53) (Figure 2A). (2) Tax associates with upstream activating kinases MEKK-1 and Tak-1 that phosphorylate IKKγ/NEMO (54-55) (Figure 2B). (3) Tax is also able to block the activity of the protein phosphatase 2A (PP2A), which dephosphorylates IKKγ/NEMO (56). (4) Tax can stimulate IKKα and IKKβ kinase activities through direct interaction (57). (5) Finally, Tax targets IκB to degradation by bridging the interaction between IκB and two subunits (HsN3 and HC9) of the 20S proteasome (58-59) (Figure 2C). Thus, Tax is able to induce a cascade of events leading to the nucleo-cytoplasmic translocation of the NF-κB subunits. As a result, infection by HTLV-1 induces a chronically persistent activation of NF-κB, causing deregulated expression of a large array of cellular genes that govern normal growth-signal transduction, such as cytokines and growth factors (IL-2, IL-6, IL-15, TNFα, and GM-CSF), cytokine receptors (IL-2 and IL-15 receptor α chains), proto-oncogenes (c-Myc), and antiapoptotic proteins (Bcl-xL). In particular, upregulation of IL-2 and IL-2 receptor α chain expression, as well as IL-15 and IL-15 receptor α, initiates an autostimulatory polyclonal expansion of T cells during the early phase of HTLV-1 infection. The persistent activation of NF-κB by Tax contributes to the initiation and maintenance of the malignant phenotype. In transgenic mice expressing Tax, elevated levels of NF-κB activity are absolutely required for the continued growth of tumor cells *in vivo* (60).

NF-κB activation also plays a role in the inhibition of the tumor suppressor p53. Inactivation of p53, through mutations or protein interactions, is common in human cancers. It was demonstrated that expression of the HTLV-1 Tax protein was sufficient for stabilization and transcriptional inactivation of wild-type p53 (61). Tax inhibits p53 transcriptional activity through the NF-κB signaling pathway, specifically the p65/RelA subunit (62, 63). Upon NF-κB activation by Tax via IKKβ, p65/RelA subunit binds to p53 and inactivates its transcriptional functions (64). NF-κB activation thus likely links the expression of Tax to T-cell transformation in HTLV-1 infected cells.

4.2. The SRF pathway

Tax protein activates the expression of cellular immediate early genes controlled by the serum response element (SRE), which contains both the serum response factor (SRF) binding element (CArG box) and the ternary complex factor (TCF) binding element (Ets box) (65). To activate this pathway, Tax does not directly bind to the CArG box but directly interacts with SRF (66) and TCF (67) transcription factors. In addition, Tax interactions with CBP/p300 and PCAF are essential for activation of SRF-mediated transcriptional activation (67) (Figure 3). Dimeric transcription factors such as AP-1 and Egr-1 that result from the activation of the SRF pathway, are highly expressed in HTLV-1 infected T-cells (68), and regulate the expression of multiple genes essential for cell proliferation, differentiation and prevention of apoptosis. In addition to Tax, HTLV-1 HBZ protein also plays a regulatory role in the SRF pathway. Indeed, HBZ directly binds to c-Jun and JunD AP-1 proteins (69-70) and may deregulate the human telomerase catalytic subunit (hTERT) in the late stages of leukemogenesis (71**)**

5. CONCLUSION

HTLV-I was the first human retrovirus associated with human disease (1). After transmission of HTLV-I, 2–5% of carriers are likely to develop adult T cell leukemia (ATL) after a long latent period. The molecular mechanisms that govern oncogenesis and other pathologies induced by HTLV-1 have been the focus of investigations and have allowed dissecting many cellular processes. However, the pathogenesis of ATL remains completely understood and there are no effective therapies for the disease. Because of their limited genome size (8,506 bp for HTLV-I), complex retroviruses have evolved to use RNA splicing to express regulatory genes with pleiotropic actions. Among them, Tax is thought to play a central role in leukemogenesis through its multiple protein interactions and deregulation of cellular pathways. Tax transgenic mice generated using the Lck proximal promoter to restrict transgene expression to developing thymocytes, developed diffuse large-cell lymphomas and leukemia with pathological and immunological features characteristic of acute ATL (60). This recent study suggested that Tax expression alone is sufficient to induce ATL and provided a unique animal model for developing new therapeutic strategies. However, since Tax is a major target of the host immune system (see review by Bangham *et al.* in this issue), its expression is often lost in ATL cells, indicating that Tax is dispensable in the last phase of leukemogenesis. Little is known about the functions of proteins encoded by the other pX genes (Rex, p12I, p13II, p30II and HBZ). Recent data indicate that p30II protein modulates cell cycle and apoptosis regulatory genes (72). As a result, p30II may play a role in T-cell survival and promote cell-to-cell spread of HTLV-1 (73). During the late stages of ATL, the HTLV-I bZIP factor (HBZ), which is probably the only viral product expressed in all ATL cells (74-75), may support proliferation and growth of ATL cells. The outcome of HTLV-1 infection is thus influenced by different cellular and viral regulatory factors playing various roles at different phases of leukemogenesis (Figure 4). The challenge is to identify all molecular interactions between the human proteome

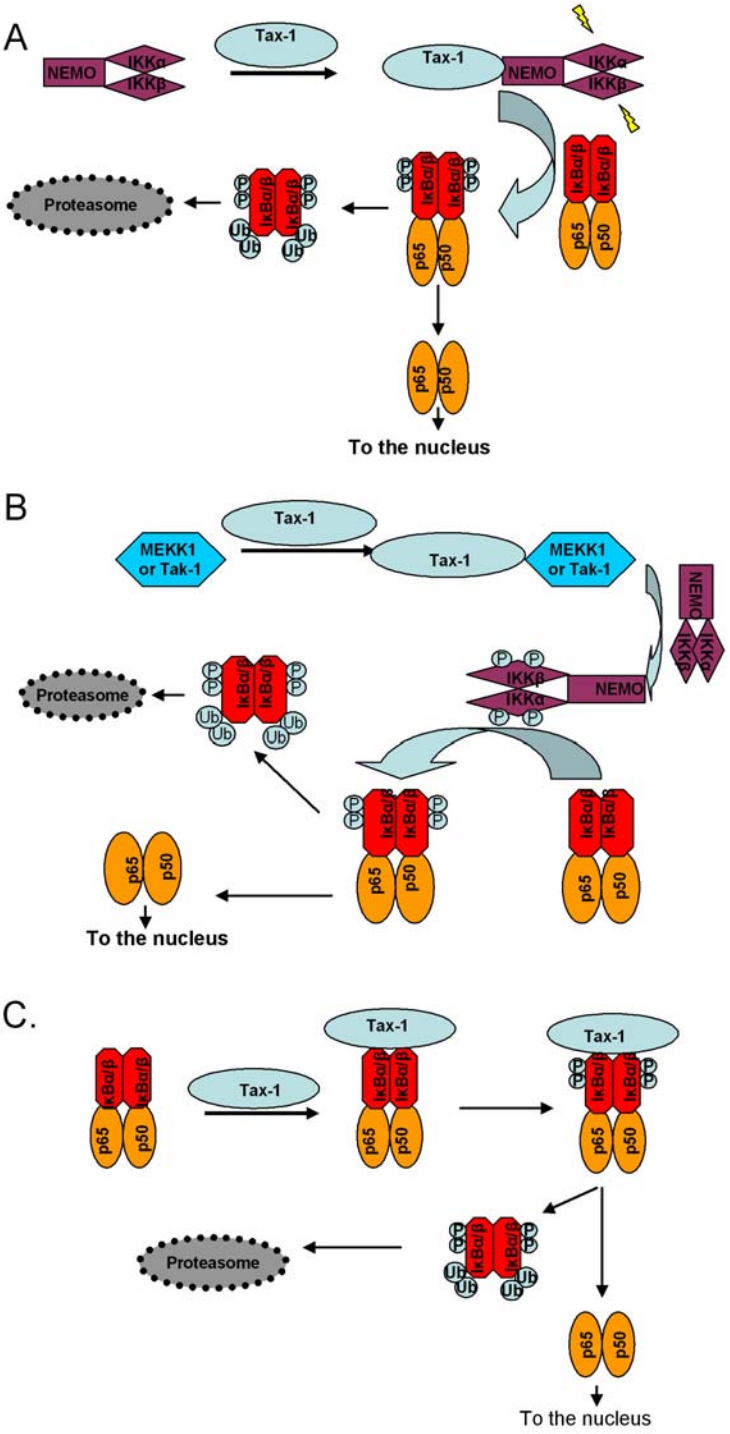

Figure 2. Schematic illustration of the NF-κB pathway activation by Tax. A. Tax activates IKKα and IKKβ kinases by interacting with NEMO. IKKs phosphorylate and induce the degradation of IκBs. Free NF-κB subunits enter the nucleus and regulate transcription of several genes. B. Tax interacts with upstream kinases of IKKs, MEKK1 and Tak-1. This interaction induces their activation and phosphorylation of IKKs kinases. Activated IKKs phosphorylate and induce the degradation of IκBs. Free NF-κB subunits enter the nucleus and regulate transcription of several genes. **C.** Tax activates the NF-κB pathway by directly interacting with IκBs. This interaction induces phosphorylation and degradation of IκBs. Free NF-κB subunits enter the nucleus and regulate transcription of several genes.

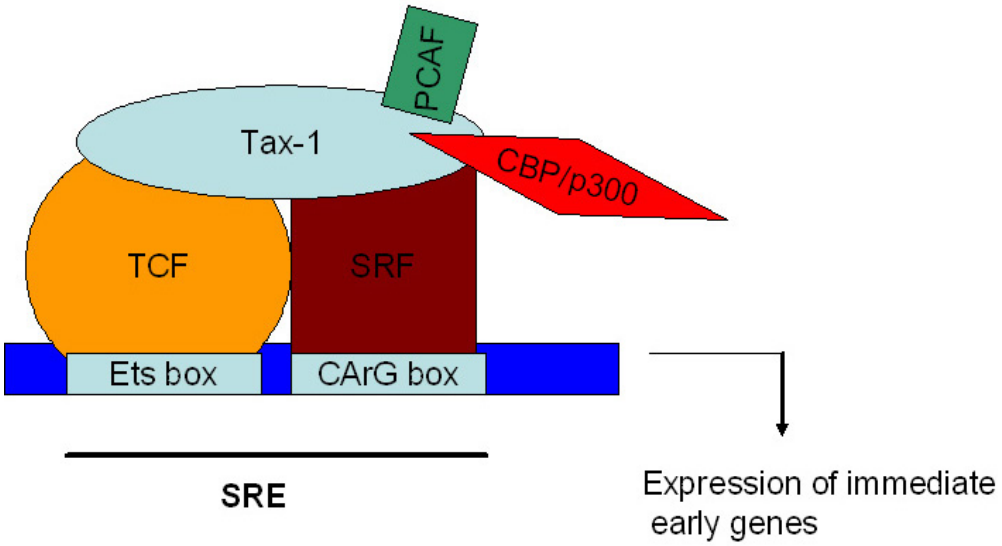

Figure 3. Schematic illustration of the DNA elements, activators and co-activators involved in Tax-induced transcriptional activation of SRF-dependent promoters. Tax interacts with SRF and TCF factors and activates the expression of immediate early genes leading to the expression of AP-1 transcription factors.

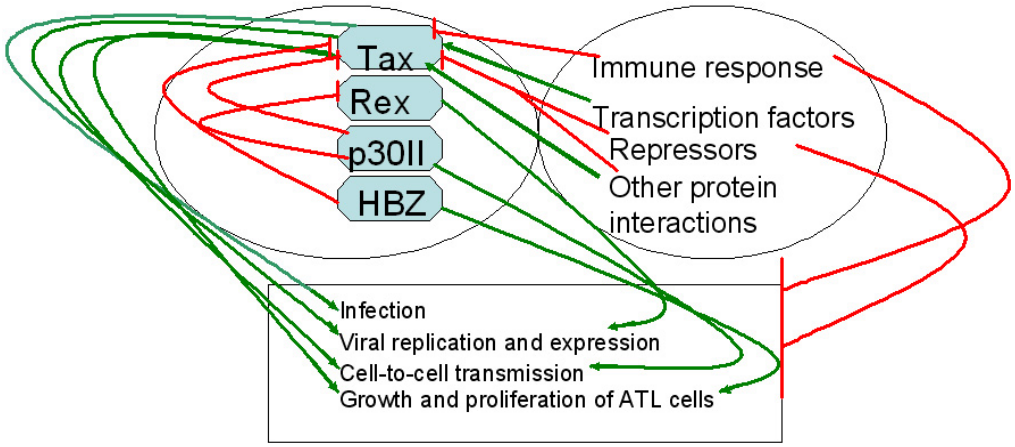

Figure 4. Model summarizing current knowledge of the functional interplay between HTLV-1 and host cell regulatory proteins to induce ATL.

and HTLV-1 viral products, to integrate those data in a dynamic view of the ATL induction and to generate new hypotheses that would represent specific features of HTLV-1 pathogenesis. The recent sequencing of the human genome (76) has created tremendous opportunities and has led to new approaches and tools for understanding human molecular systems and associated diseases. Technically, the mapping of the human genome has provided highly evolved tools that now allow automation of many biological research processes and enable laboratories to conduct large-scale, high-throughput testing and profiling. Thus, researchers in the HTLV field should adopt integrative systems biology approaches rather than focusing on individual HTLV proteins. We believe that such multi-disciplinary approach is needed to understand the complex labyrinth of connections between HTLV-1 and human T-cell proteomes.

6. ACKNOWLEDGEMENTS

This work was supported by the *Fonds National de la Recherche Scientifique* (FRS-FNRS). SL, MB, JFD, FD, RK and JCT are members of the FNRS.

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Key Words: Protein Interactions, HTLV-1, Tax, HBZ, Review

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