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HTLV-1 Yin and Yang: Rex and p30 Master Regulators of Viral mRNA Trafficking

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Abstract

Human retroviruses are associated with a variety of malignancies including Kaposi's sarcoma and Epstein-Barr virus-associated lymphoma in HIV infection, T-cell leukemia/lymphoma and a neurologic disorder in human T-cell lymphotropic virus type 1 (HTLV-1) infection. Both HIV and human T-cell lymphotropic virus type 1 have evolved a complex genetic organization for optimal use of their limited genome and production of all necessary structural and regulatory proteins. Use of alternative splicing is essential for balanced expression of multiple viral regulators from one genomic polycistronic RNA. In addition, nuclear export of incompletely spliced RNA is required for production of structural and enzymatic proteins and virus particles. Decisions controlling these events are largely guarded by viral proteins. In human T-cell lymphotropic virus type 1, Rex and p30 are both nuclear/nucleolar RNA binding regulatory proteins. Rex interacts with a Rex-responsive element to stimulate nuclear export of incompletely spliced RNA and increase production of virus particles. In contrast, human T-cell lymphotropic virus type 1 p30 is involved in the nuclear retention of the tax/rex mRNA leading to inhibition of virus expression and establishment of viral latency. How these two proteins, with apparently opposite functions, orchestrate virus replication and ensure vigilant control of viral gene expression is discussed. **(AIDS Rev. 2008;10:195-204)**

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Key words

HTLV-1. Rex. p30. RNA export. Posttranscriptional regulation.

Introduction

In 1977, epidemiologic studies revealed the presence of unusual clusters of adult T-cell leukemia/lymphoma (ATLL) in contained areas of Japan, suggesting that a transmissible agent may be involved in the disease[1]. The first description of the virus was made after the discovery of the human T-cell growth factor (interleukin-2; IL-2), allowing long-term *in vitro* culture of T-cells and establishment of T-cell lines from a patient with a cutaneous T-cell lymphoma[2,3]. A retrovirus was identified as the etiologic agent of ATLL and the terminology human T-cell lymphotropic virus type 1 (HTLV-1) was adopted[4]. Adult T-cell leukemia/lymphoma is an aggressive and fatal malignancy of $CD4^+$ T lymphocytes. The overall survival with various chemotherapy regimens is poor, ranging between 5.5 and 13 months in several cohorts of patients presenting predominantly with acute leukemia or lymphoma[5]. The HTLV-1 infection is also associated with a chronic and progressive neurologic disorder named HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP)[6,7] as well as other malignancies such as HTLV-1-associated arthropathy, HTLV-1-associated uveitis, infective dermatitis, and polymyositis[8].

The low incidence and the long latency of HTLV-1-associated ATLL suggest that, in addition to viral infection, accumulation of genetic defects is required for cellular transformation *in vivo*. The HTLV-1-mediated T-cell transformation presumably arises from a multistep oncogenic process in which the virus induces chronic T-cell proliferation, resulting in the accumulation of genetic defects,

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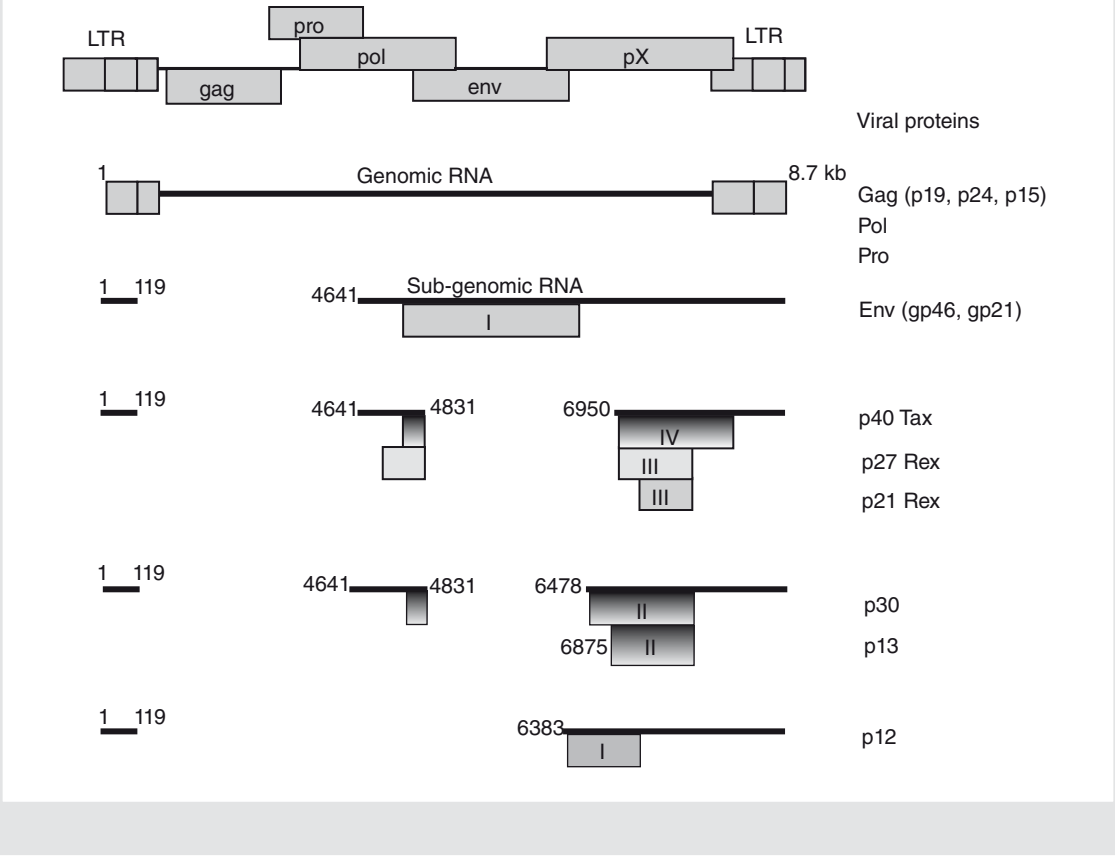

Figure 1. *Expression of HTLV-1 proviral genome. The genome of HTLV-1 encodes common structural and enzymatic proteins found in retroviruses (Gag, Pro, Pol and Env) and multiple regulatory and accessory proteins. Expression of HTLV-1 proviral genome is regulated by differential splicing of the single genomic mRNA, producing unspliced, singly spliced, and multiply spliced mRNA. LTR: long terminal repeat.*

deregulated growth, and expanded lifespan of infected cells[9]. Profound differences exist in the pathogenesis of ATLL and HAM/TSP. The ATLL is characterized by a monoclonal expansion of virus-infected cells. The virus is latent and viral gene expression is barely detectable, with both the cytotoxic T-lymphocyte (CTL) and antibody immune responses against the viral antigens being poor. In contrast, in HAM/TSP, expression of viral genes is higher, leading to *de novo* infection and polyclonal expansion of infected cells. In these patients, a strong CTL and antibody response is present, causing a constant killing of infected cells, which is balanced by *de novo* infection[10,11]. The determinants that lead to the development of ATLL versus HAM/TSP are unclear.

The genome of HTLV-1 encodes common structural and enzymatic proteins found in retroviruses (Gag, Pro, Pol, and Env). In addition, as a complex retrovirus, HTLV-1 encodes multiple regulatory and accessory proteins in four overlapping open reading frames located in the pX region of the viral genome[12,13] (Fig. 1). Expression of the various open reading frames is controlled by differential splicing of the single genomic mRNA, producing unspliced, singly spliced, and multiply spliced mRNA[15]. Therefore, the replication of HTLV-1 is controlled by a group of nuclear and cytoplasmic processes, including transcription, splicing, mRNA nuclear export, RNA stability, and translation. In addition, recent studies suggest the existence of viral encoded RNA from the 3' long terminal repeat (HBZ)[17], which are also subject to alternative splicing and generate additional viral proteins.

The HTLV-1 gene expression is self-regulatory, determined by the expression of two viral-encoded regulatory genes that act in trans at the transcriptional (tax) or post-transcriptional level (rex). Virus expression is controlled by the 5' long terminal repeat (LTR). While the HTLV-1 LTR can be activated by numerous cellular factors, potent activation is achieved after recruitment of the viral Tax protein, which brings together the cellular factor, CREB, and the transcriptional co-activators, CBP/p300 and PCAF, to the transcription machinery[18-23]. Tax is a relatively small protein with a remarkable number of distinct functions, including cellular proliferation, inhibition

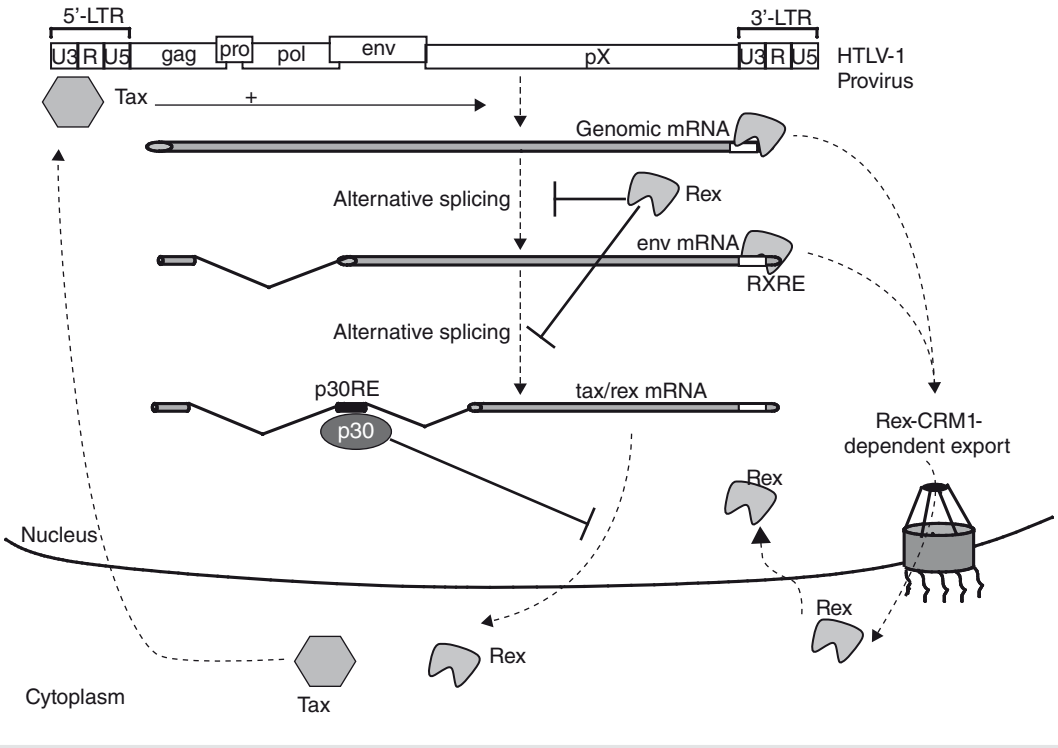

Figure 2. *Control of viral mRNA trafficking by Rex and p30. Unspliced and singly spliced mRNA are exported to the cytoplasm by the Rex-CRM1 nuclear export pathway. The doubly spliced tax/rex mRNA is retained in the nucleus by p30. RXRE; Rex responsive element; CRM: chromosome maintenance region.*

of apoptosis, genetic instability, telomerase activation, and inactivation of tumor suppressors[24-28]. Tax is expressed from the *tax/rex* RNA which also encodes for the Rex protein. Thus, in contrast to the Tat and Rev proteins of HIV, HTLV-1 Tax and Rex are co-regulated and expressed from the same RNA.

HTLV-1 Rex: a positive posttranscriptional regulator

RNA nuclear export adaptor

Cellular RNA processing leads to rapid splicing of intronic sequences or their eventual destruction, which contrasts with the need for unspliced and partially spliced mRNA required for HTLV-1 replication. The HTLV-1 and other related virus (HIV) have evolved a molecular mechanism in which viral adaptors (Rex for HTLV-1 and Rev for HIV-1) are recruited to a specific cis-regulatory RNA sequence. Several studies have demonstrated that Rex can interfere with the cellular splicing machinery and lead to cytoplasmic accumulation of unspliced genomic RNA encoding for GAG, PRO, POL, and singly spliced RNA encoding for Env (Fig. 2)[29]. Therefore, once the viral adaptors are recruited onto the viral RNA, they inhibit the recruitment of retention and splicing factors and export the RNA to the cytoplasm as mature RNA. In general, proteins cargos are transported through the nuclear membrane by a set of cellular receptors known as exportins and importins that recognize specific sequence in their proteins cargos. Proteins only having a nuclear localization signal (NLS) are selectively imported into the nucleus. However, proteins having an NLS and NES (nuclear export signal) are recognized by both receptors and then shuttles between nucleus and cytoplasm. Similar to HIV Rev[30], HTLV Rex has evolved a NES, which, with its NLS, allows shuttling between the nucleus and cytoplasm and stimulates the nucleocytoplasmic export of not fully spliced viral RNA through recruitment of chromosome maintenance region 1 (CRM1)/(or Exportin1) (Fig. 2). The NES domain, known as the Rex-activation domain, is a short, amino acid sequence (LSAQLYSSLSLD), highly hydrophobic, with leucine residues interspersed with hydrophilic residues[31]. The activation domain of Rex was shown to complement a defective effector domain mutant of Rev and was

dependent on the hydrophobic nature of the leucine residues[32]. Mutations of the four leucine residues within the Rex-activation domain demonstrate that they are critical for nuclear export of mRNA and this interaction requires a hydrophobic region[32,33]. A core tetramer sequence (LXLX), along with two upstream hydrophobic residues (in the case of Rex and Rev, two essential leucines), compose the Rex NES[32]. Human CRM1 is an export receptor for leucine-rich NES and was initially found to be a receptor for the Rev NES[34-36]. The CRM1 uses guanosine diphosphate/guanosine triphosphate (GDP/GTP) guanine nucleotide exchange of the GTPase Ran to function. The CRM1 binds to RanGTP, along with a NES-containing protein, and translocates this complex across the nuclear pore where RanGTP is then converted to RanGDP. The regulator of chromosome condensation 1 (RCC1) catalyzes the GTP to GDP conversion[37]. Rex was also shown to bind to the CRM1 receptor in cooperation with RanGTP and RanBP3, a scaffold protein that aids in the formation of the protein-export complex, in addition to binding to RCC1 to promote more efficient Ran nucleotide exchange[38,39]. RanBP3 was shown to stabilize the RanGTP-CRM1-RanBP3-NES complex in the nucleus.

Several studies have also demonstrated that the formation of Rex-Rex multimers on the Rex target sequence is critical for HTLV-1 nuclear export of mRNA. Other Studies with HIV Rev demonstrated that multiple copies of Rev were required to bind to the Rev responsive element (RRE) in order to accumulate cytoplasmic HIV mRNA[40]. Furthermore, mutations of Rex and Rev that failed to form multimers act as dominant-negative mutants, suggesting that multimerization is critical for the functions of these proteins[41]. Although multimerization was found to be critical for Rex function, Rex mutants defective for multimerization retained their ability to shuttle in and out of the nucleus[42]. The importance of Rex multimerization was highlighted using an *in vivo* rat model. In fact, HTLV-1 infection of rats produces high levels of *tax/rex* mRNA, but fails to produce significant expression of Gag and Env proteins[43]. In these models, the activity of Rex has been found to be quite low compared to that in human cells and this failure was due to the rat CRM1. Unlike human CRM1, rat CRM1 could hardly support Rex function because of its poor ability to induce Rex-Rex multimerization[44]. However, establishment of a transgenic rat with human CRM1 produced 100-1,000-fold more HTLV-1 than did T-cells derived from wild-type rats[45]. In addition to functioning as a Rex exporter, CRM1 serves as an inducing factor for Rex multimerization on mRNA by aiding in the complex formation required for HTLV-1 mRNA export[39,46]. Residues 411 and 414 of CRM1 were critical for Rex (but not Rev) multimerization, a region distinct from the role of CRM1 in the export of Rex. These two domains (along with residues 474 and 481) overlap with the region required for efficient binding of CRM1 to the scaffold protein RanBP3, suggesting that the multimerization of Rex and the interaction with RanBP3-CRM1 are linked[46]. Collectively, the requirement for multiple bound copies of Rex on the mRNA is believed to protect HTLV-1 mRNA from being bound by proteins that could induce cellular splicing or nuclear retention. An increased number of Rex proteins would also facilitate binding of a larger number of CRM1 proteins, allowing for more efficient nuclear export. Studies have also shown that translation-initiation factor eIF-5A may play a part in the formation of the Rex homo-oligomers[47].

RNA binding protein

Rex is known to be a nuclear/nucleolar phosphoprotein acting at the posttranscriptional level (Fig. 3A). In addition to the activation domain, Rex function is mediated through direct interaction with a cis-acting RNA element termed the Rex-responsive element (RXRE)[48], present in the 3' LTR (Fig. 3B). The RXRE forms a distinct stem-loop structure that is orientation dependent, but position independent, and is separate from 3'-mRNA processing and polyadenylation[49]. Rex function requires this large stem-loop and a 10-nucleotide sub-region within the RXRE of viral transcripts, which directly interacts with the Rex protein[50-52]. Rex shares many features with its homolog HIV Rev. While Rex and Rev have very little sequence homology, they both are functionally and structurally similar. In fact, Rex and Rev both have an arginine-rich region that is responsible for binding to their respective response elements; however, only Rex can functionally substitute for Rev[51,53-55] using a Rev-deficient HIV-1 provirus[55]. Rex can bind to both responsive elements, whereas Rev is unable to recognize the RXRE[54]. The minimum continuous regions within the RRE that are required for Rex and Rev functions differ between the two proteins, with Rex requiring a longer region of the RRE to bind (289 nt to 204 nt)[56]. Rex also has a higher affinity for RXRE than RRE, and regulates viral mRNA expression to a much higher extent with the RXRE. In contrast to the interaction between Rev and its responsive element, Rex's interaction with its RXRE has also been found to be inhibited by the cellular protein, heterogeneous nuclear ribonucleoprotein A1[57]. Another difference between Rex and Rev is that in HIV, the RRE is only found in unspliced or incompletely spliced HIV-encoded mRNA. However, all HTLV-1 mRNA carry the RXRE at their 3' end. While the reason for this remains unclear, one possible explanation is that Rex binding to the RXRE stabilizes a complex secondary structure that bridges the poly-A signal and the poly-A site together since these are unusually far apart in the HTLV-1 genome (Fig. 3B). In fact, it has been proposed that the RXRE plays a role in the polyadenylation of HTLV-1 transcripts[58]. The structure of the stem-loop allows the AAUAAA hexamer and GU-rich elements to come into close contact, which then serves as a signal for polyadenylation. The Rex NLS[59] binds to the nucleolar shuttling protein B-23, importing Rex into the nucleolus[60]. Mutations of the arginine

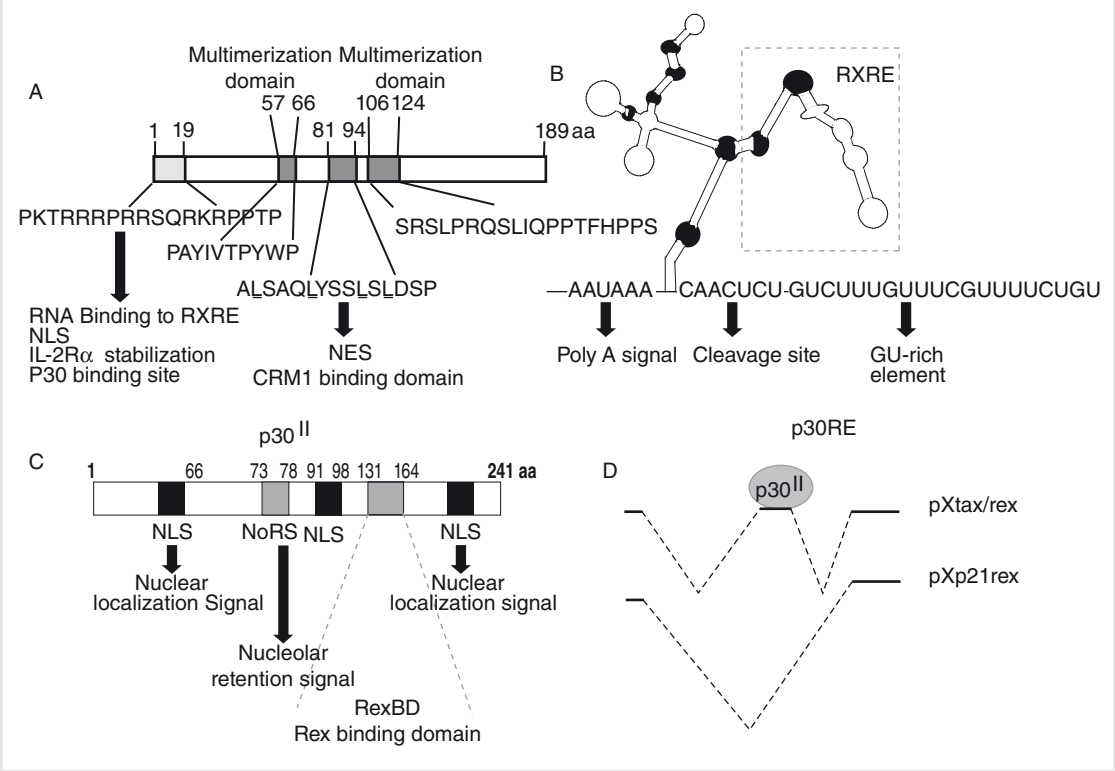

Figure 3. *Rex and p30 functional domains and structures. Mapping of Rex **(A)** and p30 **(C)** functional domains and their respective target sequences **(B)** and **(D)**: the RXRE RNA responsive element; p30 specific responsive element p30RE. LTR: long terminal repeat; RXRE; Rex responsive element; CRM: chromosome maintenance region.*

residues contained in this domain prevent Rex from nuclear accumulation and from binding to RXRE sequences[54]. Interestingly, the arginine-rich region has recently been found to be involved in Rex-p30 interactions[61].

In addition to its role in RNA trafficking, Rex has been shown to increase mRNA stability and/or enhance the translation of incompletely spliced mRNA, but the exact mechanism(s) underlying this activity is still not understood[62]. Rex activities have been shown to be regulated by phosphorylation since treatment of an HTLV-1 infected cell line with a protein kinase inhibitor blocked the cytoplasmic accumulation of unspliced *gag-pol* mRNA[63]. Phosphorylation of serine 70 in Rex appeared to be dependent on cellular protein kinase C[64]. Rex was also found to be phosphorylated on serine 177 and threonine 174. The exact role of Rex phosphorylation still remains to be determined. In addition to Rex's role in regulating viral gene expression, Rex has been shown to affect cellular gene expression. Rex was able to augment Tax-mediated increases in IL-2 expression and stabilize the IL-2 receptor-alpha chain (IL-2Rα) mRNA[65,66]. Rex was able to prolong the half-life of *IL-2Rα* mRNA, independent of nuclear-cytoplasmic transport[66]. The N-terminal, basic amino acid portion of Rex, encoding the NLS signal, was found to stabilize the 201-300 base pair region in the 5'-coding region of the *IL-2Rα* mRNA[67,68]. In this manner, Rex may contribute to cellular transformation of HTLV-1 infected cells. Rex has also been shown to increase *FynB* (p59fyn) expression, possibly by affecting FynB splicing[69]. FynB is a src family protein-tyrosine kinase that regulates T-cell receptor stimulation[70]. Co-transfection experiments in Jurkat T-cells demonstrated that Rex could augment Tax in increasing vascular cell adhesion molecule-1 (VCAM-1) and lymphocyte function-associated antigen-3 (LFA-3) expression[71]. Both VCAM-1 and LFA-3 are important proteins in T-cell adhesion, aiding in proliferation of uninfected cells and virus spread.

Although considerable knowledge about the role of Rex in virus replication has been gained, important questions still remain as to how Rex is able to regulate some viral mRNA but not others since all HTLV-1 transcripts contain an RXRE sequence. If both Tax and Rex act as positive regulators of viral gene expression, how is the virus able to reduce its expression and evade immune defenses? Answers to these questions came in 2004 when a negative viral posttranscriptional regulator was described. The

study demonstrated that p30 binds to and subsequently retains the *tax/rex* message in the nucleus, preventing production of viral structural proteins by Tax and Rex[72].

HTLV-1 p30: a negative posttranscriptional regulator

In the last decade several investigators have started to uncover the functions of additional HTLV-1 accessory proteins encoded by the pX region (p12, p13, and p30)[73-75]. Although it has been proposed that most of these proteins are dispensable for *in vitro* human T-cell immortalization[67,76,77], in our experience such cell lines have multiple growth defects *in vitro*. Nonetheless, several aspects about these small regulatory proteins are irrefutable. They are all expressed *in vitro* and RNA can be detected in HTLV-1 transformed T-cell lines[78-80]. They are also expressed *in vivo* since both cell-mediated and antibody immune responses directed against these proteins have been detected in infected patients[81,82]. These regulatory proteins play critical roles for *in vivo* infectivity in a rabbit model[83-87]. Of note, these genes are located in the originally called "non-conserved" region of the HTLV-1 genome, which has the highest genetic variability among isolates. Although isolates with stop codons in these genes have been found in infected patients, it does not mean that these genes were originally mutated during initial infection and/or the early stages of disease. These genes may have already accomplished their functions prior to being mutated. In fact, isolates with a stop codon in the *tax* open reading frame have been reported. Recent reviews have described in detail the functions of the p12 and p13 proteins[73,74]. The p30 protein is intriguing because it is reported to act as both a transcriptional and posttranscriptional regulator, a characteristic shared by another nucleolar resident protein, nucleolin. The p30 has similarities with serine-rich proteins, including several transcriptional activators such as oct-1, oct-2, pit-1, engrailed, and POU-Mi transcription factors[13]. Transient transfection experiments confirmed the transcriptional activity of p30 in Gal4 experiments. In addition, DNA microarray studies also showed that p30 was able to modify the expression profile of key cellular genes involved in leukemogenesis[88]. The transcriptional activities of p30 have been reviewed[73,74]. This review focuses on the posttranscriptional activity of p30 and the interplay with Rex.

The p30 RNA response element (p30RE) is responsible for the formation of the ribonucleoprotein complex and the retention of the *tax/rex* RNA in the nucleus, reducing Tax and Rex protein expression and promoting latency[72] (Fig. 2). Similar results were subsequently obtained using p28, a p30 homolog, in HTLV-2[89]. This is a unique example of negative regulation among viruses. Although the exact sequence required for p30-mediated retention has not yet been defined, earlier studies have shown that the p30RE encompass the envelope exon junction since the *tax/rex* but not the viral RNA is regulated by p30 (Fig. 3D)[72]. The p30 contains a sequence with homology to a NES, but heterokaryon assays demonstrated that, unlike Rex, p30 is a non-shuttling protein[72]. In addition, p30 does not interact with CRM1 and a mutation, which restores the consensus NES sequence (Ser to Leu), does not provide shuttling capability to p30 (unpublished data).

The nucleolus is not membrane bound and its structure is maintained by an accumulation of ribosomal RNA and proteins such as nucleolin or protein B23. Most proteins are only transiently retained in the nucleolus through protein or RNA interactions, and proteins with longer resident times usually harbor specific signals[90]. These signals tend to be nucleolar retention signals, generally characterized by sequences rich in arginine and lysine[91,92]. *In vivo* kinetics of p30 fused to a green fluorescent protein (GFP-p30) in the nuclear and nucleolar compartments, analyzed by live cell imaging, indicated that p30 is highly mobile in the nucleus but very static in the nucleolus[93]. In cells expressing GFP-p30, only a small fraction of the fluorescence signal was lost within the first five seconds after a bleaching event, followed by a slower decline of the nucleolar signal. Surprisingly, the kinetics of GFP-p30 are very similar to nucleolar methyltransferase fibrillarin-GFP, suggesting that p30 is tightly bound to nucleolar components[93]. The nucleolar retention signal of p30 was identified as an unusually short Arg-rich stretch, RRCRSR, (Fig. 3C) and deletion of this sequence or replacement of all Arg's into Ala's prevents accumulation of p30 in the nucleolus[93]. Single substitution of any Arg had no effect on nucleolar localization. In the same study, localization of various truncated forms of p30 revealed the presence of four NLS domains in p30 (Fig. 3C).

The nucleolus is critically involved in the control of the cell cycle, DNA repair, aging, and mRNA export[94]. Many viruses encode for nucleolar proteins, which are involved in the replication of viral genomes, as well as in the transcriptional and posttranscriptional regulation of gene expression[95,96]. The use of mutants of p30 unable to accumulate into the nucleolus indicated that nucleolar localization of p30 is dispensable for its transcriptional and posttranscriptional effects[93]. Nucleoli are mainly the sites of ribosome biogenesis, a highly complex process leading to the production of pre-ribosomal particles, which are then released into the nucleoplasm and exported to the cytoplasm as mature ribosomal subunits. Confocal microscopy indicated that a GFP-p30 fusion protein localized to the granular center[93], which is the site of pre-ribosome assembly. Interestingly, p30 was also found to interact with L18a, a constituent of the 60S ribosomal subunit[93]. While the biological significance of this interaction is unclear, it is worth noting that L18a interacts with viral proteins and with initiation factor eIF3 and facilitates internal re-initiation of translation in Cauliflower mosaic virus- and Hepatitis C virus-infected cells[97-101]. The p30 encoding mRNA completely

overlaps two other viral gene's open reading frames, namely p12 and p13. Whether p30 expression may directly or indirectly modulate internal initiation to increase expression of these genes warrants further studies.

The accumulation of several proteins in the nucleoli including Rex and Rev is transcription-dependent[102,103]. Measurement of GFP-p30 recovery kinetics in response to actinomycin D treatment by fluorescence recovery after photo-bleaching indicated a much slower recovery of fluorescence following actinomycin D treatment[93]. This confirms that GFP-p30 is in fact retained in the nucleoli in a transcription-dependent manner via interaction with less-mobile nucleolar components. This was supported by the calculated T1/2 recovery for the wild-type GFP-p30 (approximately 9 seconds) compared to GFP-p30 in cells treated with actinomycin D (77 seconds). These results indicate that p30 retention is associated with RNA polymerase II- and RNA polymerase III-dependent transcription of nascent RNA[93].

The p30-mediated repression of *tax/rex* RNA export does not depend upon the splicing machinery, since cDNA from the *tax/rex* sequence remained sensitive to p30 inhibition[72] and p30 was found to interact with the spliced *tax/rex* mRNA in transfected cells[72,89]. Since the p30RE is not present in viral RNA until the *env* exon has been spliced out, it is likely that p30 interacts only with fully spliced, mature *tax/rex* nuclear transcripts.

Rex and p30 interactions control the fate of viral RNA and govern the switch between virus latency and replication

Both HTLV-1 Rex and p30 are RNA binding regulatory proteins. Rex interacts with the RXRE and stimulates nuclear export of incompletely spliced viral RNA, thereby increasing production of virus particles. In contrast, p30 is involved in the nuclear retention of the *tax/rex* mRNA leading to inhibition of virus expression and establishment of viral latency. How these two proteins, with apparently opposite functions, integrate in the viral replication cycle is unclear. Recent studies shed light onto how these two proteins modulate each other's function. Using recombinant protein and transient transfection assays, p30 was found to specifically form complexes with Rex *in vivo* and *in vitro*[61]. A region located between amino acids 131-164 of p30 encompasses the Rex binding site (Fig. 3C), a region not present in the p13 protein, which expectedly did not bind Rex. Mutants of p30 that localized in the nucleus or the nucleolus were both able to interact with Rex, suggesting that these proteins form complexes in the nucleoplasm. Interactions between Rex and p30 were strengthened when co-expressed in the presence of an HTLV-1 molecular clone but not in presence of Tax, suggesting that p30-Rex complexes are stabilized onto viral mRNA[61]. This was further confirmed in *in vitro* experiments in which viral RNA incubated with Rex and p30 increased complex formation, while RNAse had the opposite effect. Complexes of p30 and Rex also assembled onto cellular RNA, since the presence of RNAse inhibitor in absence of viral RNA was sufficient to stabilize p30-Rex interactions[61]. A Rex mutant in which six arginines involved in RNA binding have been mutated to lysines (RexLys) was shown to have the same cellular localization as the wild-type Rex, but to be selectively defective in RNA binding. Transient assays showed that RexLys failed to interact with p30 in the absence or in the presence of an HTLV-1 molecular clone, suggesting that Rex-RNA interactions may be required to recruit p30, or that p30 may interact within the RNA-binding site of Rex mutated in RexLys. Additional data suggested that p30, when bound onto RNA, efficiently recruited Rex even in the absence of an RXRE. Thus, Rex-RNA binding is not absolutely required, and consequently, p30 can interact within the Rex RNA-binding domain[61]. Studies aimed at understanding the specificity of p30 showed that Rex and p30 differentially associate onto viral mRNA. The p30-Rex complexes were significantly decreased in transient assays using a chimera RNA containing the RXRE only and no p30RE. These findings can be explained by the fact that Rex has a high affinity for its RXRE, and whenever Rex is bound to RNA, its p30 binding domain is no longer accessible, preventing p30-Rex protein complex formation. These results explain why *gag/pol* and *env* RNA are not subject to p30-mediated suppression[72]. In contrast, p30-Rex complexes increased when a chimera RNA containing both the RXRE and the p30RE was co-expressed along with p30 and Rex. However, it is likely that Rex is unable to multimerize and to recruit CRM1 in the absence of RXRE binding. Additional studies showed that an excess of Rex partially rescues *tax/rex* RNA from p30-mediated repression. Such a mechanism of regulation allows vigilant control of viral gene expression. The p30 reduces viral expression to prevent immune detection, but Rex guarantees low basal expression to produce a small amount of Tax essential for the early stages of T-cell transformation and to sustain expression of p30 (Fig. 4).

Conclusions

Human T-cell lymphotropic virus-1 has evolved multiple strategies to extend the lifespan of infected cells. This, in turn, allows accumulation of genetic mutations and, with time, disease progression. Because HTLV-1 is very immunogenic and has low variability, reducing its expression is key to virus maintenance *in vivo*. It is no surprise that HTLV-1 has evolved numerous strategies to limit its own expression. Tax is a potent transcriptional activator and very immunogenic. Hence, Tax expression needs to be tightly controlled. Tax is generally toxic, inducing multiple cell-cycle defects and apoptosis, in cells that have not yet

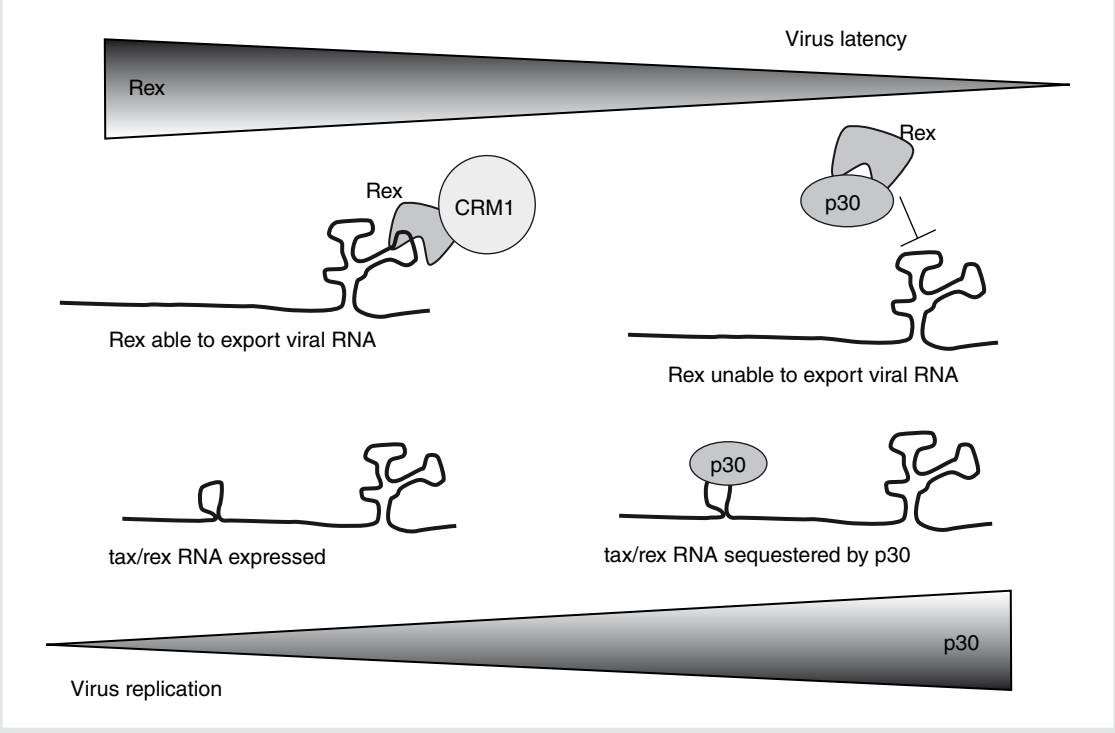

Figure 4. *Rex and p30 govern the switch between virus latency and replication. When Rex is in excess, it binds onto the RXRE and exports incompletely spliced RNA to the cytoplasm. In contrast, when p30 is in excess, it inhibits Rex expression by binding to and retaining the tax/rex mRNA in the nucleus. CRM: chromosome maintenance region.*

acquired tolerance. Following initial infection, only cells with low Tax expression are able to survive. Thus, a natural cycle exists for selection of cells with low Tax expression. The HTLV-1 p12 has been shown to reduce major histocompatibility complex class I surface expression and decrease CTL recognition of infected cells. In addition, p30 limits *tax/rex* RNA export and thereby Tax expression[75]. Surprisingly, p30 protein also affects the toll-like receptor-4 signaling pathway, suggesting that HTLV-1 subverts a component of the host innate immunity. Recently, several studies have highlighted the role of HBZ in suppression of Tax expression.

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