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MINIREVIEW

Multiple Control Levels of Cell Proliferation by Human T-Cell Leukemia Virus Type 1 Tax Protein

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Human T-cell leukemia virus type 1 (HTLV-1) is the causative agent of two diseases: adult T-cell leukemia (ATL), a fatal T-lymphoproliferative disorder, and tropical spastic paraparesis/HTLV-1-associated myelopathy (TSP/HAM), a chronic progressive disease of the central nervous system. ATL was initially identified in Japan (Uchiyama *et al.,* 1977) and is characterized by a malignant proliferation of a mature T lymphocyte with a $CD2^+$, $CD3^+$, $CD4^+$, $CD8^-$, $CD25^+$, and $HLA\text{-}DR^+$ phenotype. Leukemic cells have lobulated nuclei and are referred to as flower cells. The association of TSP/HAM with HTLV-1 infection was first reported by Gessain *et al.* (1985). TSP/HAM is characterized by a progressive demyelination of the spinal cord, but the mechanism involved in the tissue damage of the central nervous system remains unknown. HTLV-1 has also been described to be associated with other disorders such as uveitis, arthropathy, infective dermatitis, and polymyositis.

Here we review recent results concerning the involvement of HTLV-1 Tax protein in molecular events controlling cell proliferation. Taken together, these observations provide further insight into the mechanisms by which HTLV-1 induces cell transformation.

The expression of the HTLV-1 genome is controlled by two regulatory genes, the *rex* and *tax* genes, located in the 3′ region of the viral genome. Rex is a 27-kDa protein that acts at the posttranscriptional level by modulating the transport of the viral RNAs and hence by promoting the translation of unspliced and singly spliced mRNA encoding the *gag-pol* and *env* gene products, respectively (Hidaka *et al.,* 1988). Tax is a 40-kDa protein that functions in association with the activating transcription factor/CRE-binding protein (ATF/CREB) family (Zhao and Giam, 1992; Suzuki *et al.,* 1993; Adya and Giam, 1995; Yin *et al.,* 1995; Reddy *et al.,* 1997; Gachon *et al.,* 1998) to enhance viral transcription. This *trans*-activation operates through three imperfect cAMP response element (CRE)-containing 21-bp regulatory sequences in the HTLV-1 long terminal repeat (LTR). Tax also upregulates the expression of several cellular genes by activating different signaling pathways that stimulate effectors such as the serum responsive factor and the NF-κB/Rel factors (for review see Hiscott *et al.,* 1995; Yoshida *et al.,* 1995). Moreover, Tax can repress the transcription of cellular genes through factors of the basic helix-loop-helix family (Uittenbogaard *et al.,* 1994; Lemasson *et al.,* 1997).

The mode of leukemic transformation by HTLV-1 is poorly understood. HTLV-1 does not code for a known cellular proto-oncogene and has no preferential site of integration, which could explain why a transformation process is initiated. Some published results suggest that Tax not only is a transcriptional activator, but also contributes to the development of ATL. Indirect evidence for the role of Tax in leukemogenesis comes from studies on malignant cells from ATL patients, which have defective proviruses retaining the 3′-end of the HTLV-1 genome (Korber *et al.,* 1991). From experiments on primary human T-lymphocyte immortalization by a recombinant herpesvirus vector carrying the HTLV-1 3′ region (Grassmann *et al.,* 1989), it has been shown that the *tax* gene is necessary and sufficient for cell immortalization (Grassmann *et al.,* 1992). Tax can also transform established rodent fibroblasts (Tanaka *et al.,* 1990; Smith and Greene, 1991; Yamaoka *et al.,* 1992, 1996). In addition, cotransfection of Tax with Ras can also induce transformation of primary rat embryo fibroblasts (Pozzatti *et al.,* 1990). Finally, Tax transgenic mice develop several pathologies including leukemia, mesenchymal tumors, and neurofibromas (Hinrichs *et al.,* 1987; Nerenberg *et al.,* 1987; Grossman *et al.,* 1995; Coscoy *et al.,* 1998).

Modulation of different regulatory pathways and de-

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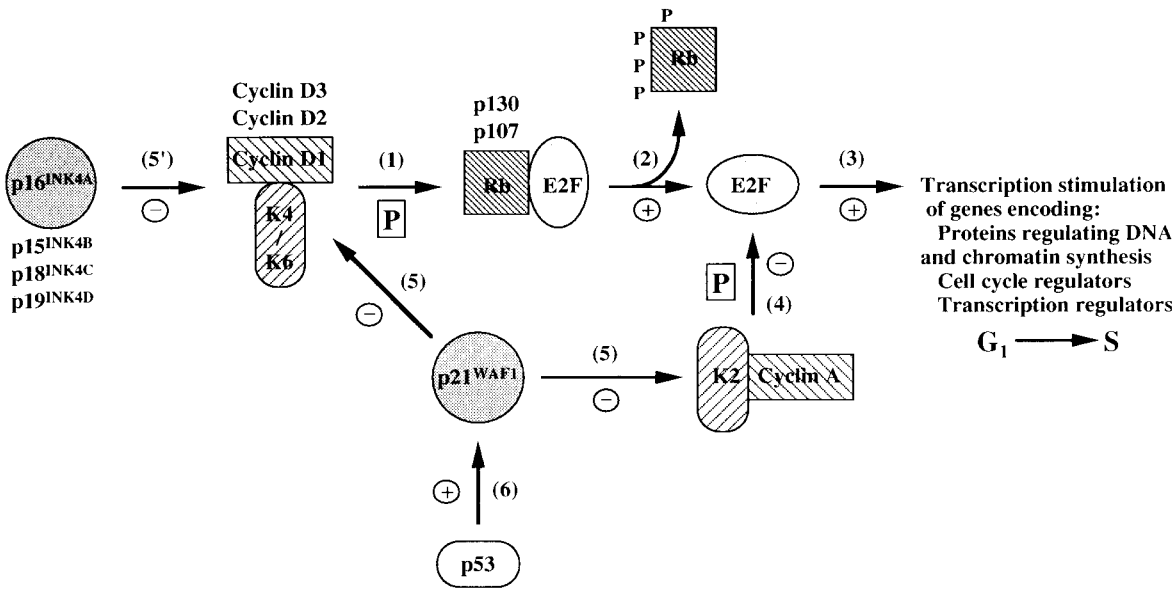

FIG. 1. Schematic representation of the role of cellular factors involved in regulation of E2F activity. This scheme includes only the cellular proteins mentioned in the paper. After mitogen stimulation, D-type cyclins (D1, D2, D3) associate with their catalytic partners, CDK4 and CDK6. Activated cyclin–CDK complexes phosphorylate the Rb protein (1). Rb is bound to E2F during G_1. Upon phosphorylation, E2F is released (2) and activates the transcription of genes required for transition into S phase (3). As E2F–DP heterodimers are differentially regulated by various members of the Rb family (see text), only E2F is shown for simplicity. This late G_1 activation of E2F declines rapidly as cells move into S phase. The reversal of E2F-mediated *trans*-activation depends on the appearance of cyclin A–CDK2 complexes, which phosphorylate the E2F1–DP1 heterodimer (4), leading to the loss of its DNA-binding activity. Cyclin A- and D-dependent kinases can be negatively regulated by specific inhibitors (5): $p16^{INK4A}$ and the p53-inducible (6) $p21^{WAF1/CIP1}$. The phosphorylation step is indicated by (P). Stimulating and inhibitory effects are shown by (⊕) and (⊖).

regulation of cellular gene expression by Tax might be implicated in cell proliferation. However, it remains unclear which of the diverse stimulation activities of Tax is essential for immortalization of HTLV-1-infected lymphocytes. Some reports suggest that NF-κB activation is essential for rodent cell transformation (Kitajima *et al.,* 1992; Yamaoka *et al.,* 1996; Matsumoto *et al.,* 1997; Coscoy *et al.,* 1998). Nevertheless, transduction in human primary T lymphocytes of a Tax mutant that is active for ATF/CREB but inactive for NF-κB results in permanent growth of the cells, suggesting that NF-κB activation by Tax is not a critical component of T-lymphocyte immortalization (Rosin *et al.,* 1998). This observation is in agreement with previously published results indicating that transcriptional activation through the ATF/CREB pathway could play an important role in Tax-mediated cellular transformation (Smith and Greene, 1991).

One possible explanation for these results is the Tax-dependent transcriptional control of the *E2F-1* gene through the ATF/CREB pathway (Lemasson *et al.,* 1998). E2F-1 is a transcription factor that belongs to the E2F family. The E2F transcriptional activity is the result of the heterodimeric association of two families of proteins, E2Fs and DPs. Six E2F proteins (E2F1–6) and two DP subunits (DP1–2) are characterized in human cells. E2F/DP factors are involved in the transcriptional regulation of genes that encode cell cycle regulatory proteins (La Thangue, 1994, 1996; Slansky and Farnham, 1996) such as (i) DNA and chromatin synthesis proteins (dihydrofolate reductase, thymidine kinase, DNA polymerase α, proliferating cell nuclear antigen, and histone H2A); (ii) cell cycle regulatory proteins (cyclin A, cyclin E, cyclin D1, p107, pRB, cdc6, hsorc1, E2F-1, and E2F-2); and (iii) cellular proto-oncogenes including c-*myc,* N-*myc, erb*-B, and B-*myb.* Thus ectopic expression of E2F-1 can lead resting cells to enter into S phase and induce cell proliferation (Johnson *et al.,* 1993; Lukas *et al.,* 1996). In addition, Tax is able to strongly potentiate the transcriptional activity of E2F-1 (Lemasson *et al.,* 1998). Mori (1997) suggests that this stimulation could be due to the enhancement of the DNA-binding activity of E2F factors by Tax as already described for the ATF/CREB family (Baranger *et al.,* 1995; Yin and Gaynor, 1996). However, it is obvious that this mechanism is not sufficient to explain the E2F activation by Tax in HTLV-1-infected cells. Indeed, E2F activity is known to be regulated by different cellular proteins whose activities, in turn, are cell cycle dependent (Fig. 1). Furthermore, Tax has mitogenic activity (Schmitt *et al.,* 1998), which regulates cell cycle progression, and probably has indirect effects on E2F transcriptional activity.

Cell cycle progression is controlled by sequential activation of cyclin-dependent protein kinases (CDK) (Van den Heuvel and Harlow, 1993; Sherr, 1994; Morgan, 1995). CDK4 and CDK6, for example, are the first to be activated after serum stimulation and are essential for the control of G_1-phase progression (Fig. 1). CDK4 and CDK6 form active complexes with D-type cyclins (D1, D2, and D3),

whose synthesis is induced by mitogenic stimulation. Activated cyclin D–CDK complexes phosphorylate the members of the retinoblastoma tumor suppressor protein family (Rb, p107, and p130) (Nevins, 1992; Helin and Harlow, 1993). In its hypophosphorylated form, Rb inhibits the transcriptional activity of E2F by formation of a protein–protein complex. Phosphorylation of Rb by cyclin D–CDK complexes releases E2Fs, enabling them to activate transcription and to stimulate cells to enter S phase.

In HTLV-1-transformed lymphocytes, the normal cell cycle is dysregulated. The observations published by Schmitt *et al.* (1998) clearly demonstrate that Tax is required to stimulate the G_1- to S-phase transition of immortalized T cells. The experiments have been carried out with primary human cord blood T cells transduced with a recombinant rhadinovirus containing the *tax* gene under a promoter whose expression is repressed by tetracycline. Suppression of Tax synthesis caused cell-cycle arrest in the G_1 phase. After removal of tetracycline, the expression of Tax stimulates the G_1- to S-phase transition of the immortalized T cells. One possible explanation for this mitogenic effect of Tax is the dissociation of the Rb–E2F complex by direct binding of Tax to Rb as already observed for other oncoviral proteins such as adenovirus E1A protein, simian virus 40 large T antigen, and papillomavirus E7. However, studies involving *in vitro* binding assays indicate that Tax does not interact with Rb directly (Low *et al.,* 1997). Another possibility is the stimulation of expression levels of CDK4, CDK6, and cyclin D isotypes by Tax. However, no transcription stimulation of the genes coding for these proteins has been observed (Suzuki *et al.,* 1996; Neuveut *et al.,* 1998; Schmitt *et al.,* 1998) although these observations conflict with the conclusions drawn by Akagi *et al.* (1996), who hypothesized that the promoter of cyclin D2 might be activated by Tax. A third possible mechanism is the activation of CDK4 and CDK6 by Tax. Indeed, in the presence of Tax, both CDK4 and CDK6 activities are stimulated (Schmitt *et al.,* 1998) and Rb is hyperphosphorylated (Neuveut *et al.,* 1998).

The activities of cyclin D–CDK complexes are regulated by specific factors called INK4 proteins (for Inhibitors of CDK4), which bind to CDK4 and CDK6 and block their kinase activities (Fig. 1). As a consequence, the cell cycle is arrested in the G_1 phase. This family of inhibitors includes $p16^{INK4A}$, $p15^{INK4B}$, $p18^{INK4C}$, and $p19^{INK4D}$ (Peter and Herskowitz, 1994; Morgan, 1995; Sherr and Roberts, 1995). Tax could activate CDK4 and CDK6 by counteracting the INK4 protein inhibitory function. As Tax binds to the ankyrin motifs of IκBα, it has been postulated that Tax could also interact with the ankyrin motif in $p16^{INK4A}$ (Suzuki *et al.,* 1996). Indeed, it has been shown that Tax forms a complex with $p16^{INK4A}$ and that this interaction interferes with the ability of $p16^{INK4A}$ to repress CDK4 kinase activity (Suzuki *et al.,* 1996; Low *et al.,* 1997). As it has been reported that the *p16^{INK4A}* gene is deleted or mutated in many human tumor cell lines (Sherr and Roberts, 1995), it may be argued that $p16^{INK4A}$ is not a relevant target for Tax *in vivo.* However, expression of the *p16^{INK4A}* gene is in fact not altered in the HTLV-1-transformed T-cell lines (Suzuki *et al.,* 1996). Therefore the functional inactivation of $p16^{INK4A}$ by Tax could play an important role in proliferation of established T-cell lines infected by HTLV-1. On the other hand, it has been demonstrated that Tax is unable to interact with other members of the INK4 family such as $p18^{INK4C}$ and $p19^{INK4D}$ (Neuveut *et al.,* 1998).

However, in Jurkat cells, which lack expression of $p16^{INK4A}$, Tax remains able to stimulate the G_1- to S-phase transition despite the absence of serum and to increase CDK4 and CDK6 activities (Neuveut *et al.,* 1998). These results suggest that Tax is able to activate CDK4 and CDK6 by a $p16^{INK4A}$-independent pathway. Neuveut *et al.* (1998) have recently found that Tax can form a complex with cyclin D3 and induces cyclin D3 phosphorylation. They proposed that binding by Tax could, through unknown processes, affect cyclin D3 phosphorylation. Phosphorylated cyclin D3 could render the cyclin–CDK complexes more active and thus stimulate cell cycle progression. However, cyclin phosphorylation has been reported to be a signal for ubiquitination and proteasome processing (Clurman *et al.,* 1996; Won and Reed, 1996; Diehl *et al.,* 1997). Indeed, phosphorylation of cyclin D1 and E on Thr286 and Thr380, respectively, regulates cyclin destruction (Won and Reed, 1996; Diehl *et al.,* 1997). Characterization of the exact site of phosphorylation seems to be essential to confirm this model. In conclusion, Tax is able to deregulate the cell cycle progression by targeting different levels, including transcription regulation as well as direct interaction with $p16^{INK4A}$ and cyclin D3. However, the link between these results obtained in established cell lines and the development of ATL still remains to be clearly demonstrated.

Cell cycle progression is also controlled by p53, which is a tumor suppressor. p53 is a transcriptional nuclear phosphoprotein activated in response to DNA damage (Fig. 2). Induction of p53 results in either cellular arrest in G_1 (mediated by the CDK inhibitor $p21^{WAF1/CIP1}$, for instance) or apoptosis (mediated by the tumor suppressor Bax, for example) (Gottlieb and Oren, 1996; Ko and Prives, 1996; Evan and Littlewood, 1998). It is therefore not surprising that the *p53* gene is the most frequently mutated gene in human cancer. Although missense mutations have been described in the *p53* gene of ATL cells, the frequency of such mutations is low and such mutations are found in only 20% of cells (Nagai *et al.,* 1991; Sugito *et al.,* 1991; Sakashita *et al.,* 1992). These observations suggest that in addition to mutation another mechanism is probably involved that acts by preventing p53 expression or function in HTLV-1-transformed T cells. Concerning the possibility that the mechanism

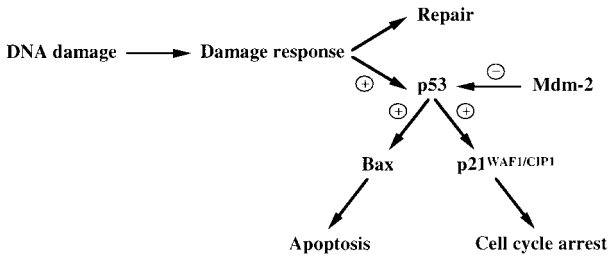

FIG. 2. Control of p53 activity. After activation by stress such as DNA damage, p53 induces transcription of genes encoding proteins that mediate either growth arrest (for instance, $p21^{WAF1/CIP1}$) or apoptosis (for example, Bax). The level of p53 is regulated through interaction with the Mdm-2 protein, which signals its degradation. Stimulating and inhibitory effects are shown by (⊕→) and (⊖→).

involves the inhibition of p53 expression, it has been proposed that Tax could repress *p53* transcription (Uittenbogaard *et al.,* 1995) as already shown for the genes encoding β-polymerase, $p56^{lck}$ kinase, and Bax (Jeang *et al.,* 1990; Brauweiler *et al.,* 1997; Lemasson *et al.,* 1997). However, elevated expressions of p53 found in several HTLV-1-transformed T-lymphocyte lines (Reid *et al.,* 1993; Yamato *et al.,* 1993; Gartenhaus and Wang, 1995) and Tax-immortalized T cells (Akagi *et al.,* 1997) do not support this hypothesis. Moreover, the half-life of p53 is increased in HTLV-1-transformed T-lymphocytes (Reid *et al.,* 1993) compared with the rapid turnover observed in normal cells. On the other hand, data obtained from transfection experiments using a p53-responsive reporter plasmid and from ionizing radiation studies demonstrate that p53 is not fully active in HTLV-1-transformed lymphocytes and that Tax alone is capable of suppressing the p53 transcriptional activity (Cereseto *et al.,* 1996; Akagi *et al.,* 1997; Mulloy *et al.,* 1998; Pise-Masison *et al.,* 1998a). In addition, Tax abrogates G_1 arrest and apoptosis induced by p53 (Mulloy *et al.,* 1998). These observations lend support to the hypothesis of functional inhibition of p53 by Tax, but the molecular mechanism by which this inhibition occurs is unknown. Some viral oncoproteins, such as adenovirus E1B 55K, simian virus 40 large T antigen, and papillomavirus E6 protein, inactivate p53 by direct binding. However, this mechanism is probably not used by HTLV-1 since no association between p53 and Tax has been characterized (Yamato *et al.,* 1993). Moreover, Tax does not interfere with the nuclear localization or DNA-binding ability of p53 (Mulloy *et al.,* 1998; Pise-Masison *et al.,* 1998b). Alternatively, Tax could regulate p53 by posttranscriptional modification. Pise-Masison *et al.* (1998b) have recently suggested that Tax could regulate p53 by increasing the ability of a kinase to phosphorylate residues Ser15 and Ser392 of p53. This hyperphosphorylated form of p53 is unable to interact *in vivo* with the TATA box-binding protein or Mdm-2 (Fig. 2), which is normally involved in p53 degradation (Pise-Masison *et al.,* 1998b). Finally, Tax could regulate p53 by an indirect effect. Indeed, Tax stimulates transcription of the viral promoter by recruiting the coactivator CREB-binding protein (CBP) (Kwok *et al.,* 1996; Giebler *et al.,* 1997; Bex *et al.,* 1998) and by interacting with basal transcription factors (Caron *et al.,* 1993, 1997; Clemens *et al.,* 1996). Thus, Tax could inhibit p53 through competition for common transcriptional regulators. Recently, Colgin and Nyborg (1998) demonstrated that Tax was able to antagonize the transcriptional activity of c-Myb by competing for CBP binding. These results confirm that such a mechanism could also be involved in the deregulation of p53 transcriptional activity by Tax.

A major part of p53-mediated growth arrest proceeds through stimulation of the CDK inhibitor $p21^{WAF1/CIP1}$, which regulates the cell cycle by inhibiting the CDKs (Fig. 1) required for progression from the G_1 to the S phase (El-Deiry *et al.,* 1993; Harper *et al.,* 1993) and also inhibits the ability of proliferating-cell nuclear antigen to activate DNA polymerase δ, resulting in inhibition of DNA replication (Waga *et al.,* 1994). However, $p21^{WAF1/CIP1}$ expression is highly stimulated in HTLV-1-transformed T-cell lines (Akagi *et al.,* 1996; Cereseto *et al.,* 1996). Although this observation may be unexpected, elevated levels of $p21^{WAF1/CIP1}$ have already been reported in transformed cells. For example, papillomavirus E7 oncoprotein-expressing keratinocytes overexpress $p21^{WAF1/CIP1}$, but E7 is able to overcome $p21^{WAF1/CIP1}$ arrest (Morozov *et al.,* 1997; Ruesch and Laimins, 1997). It has been proposed that E7 abrogates G_1/S arrest by interacting with $p21^{WAF1/CIP1}$ (Jones *et al.,* 1997), but this result is still a matter of controversy (Ruesch and Laimins, 1997). It is possible that E7 overcomes G_1/S arrest without directly affecting p21 function and acts through deregulation of E2F activity by interacting with Rb (Ruesch and Laimins, 1997). A similar control mechanism is probably involved in the case of HTLV-1 since Tax can also stimulate E2F activity (Lemasson *et al.,* 1998; Mori, 1997). The existence of an indirect mechanism is supported by the observation that Tax does not interact physically with $p21^{WAF1/CIP1}$ (Parker *et al.,* 1996; Low *et al.,* 1997). It remains intriguing, however, that Tax stimulates expression of $p21^{WAF1/CIP1}$ in infected T cells. This fact probably means that $p21^{WAF1/CIP1}$ might play an important role in the activation of T cells by Tax, one possibility being that $p21^{WAF1/CIP1}$ is involved in stimulation of NF-κB activity (Perkins *et al.,* 1997) and can cooperate with Tax to stimulate the NF-κB pathway (Parker *et al.,* 1996).

Reports on the capacity of Tax to mediate apoptosis are contradictory. Although Copeland *et al.* (1994) have found that susceptible T cells, transfected with a Tax-expressing vector or treated with soluble Tax, become less susceptible to anti-Fas-induced cell death, other investigators have demonstrated that Tax has a potential apoptotic effect (Yamada *et al.,* 1994; Chlichlia *et al.,* 1995, 1997; Fujita and Shiku, 1995; Los *et al.,* 1998). The molecular mechanism of Tax-induced apoptosis could involve Fas, a receptor on the surface of lymphocytes

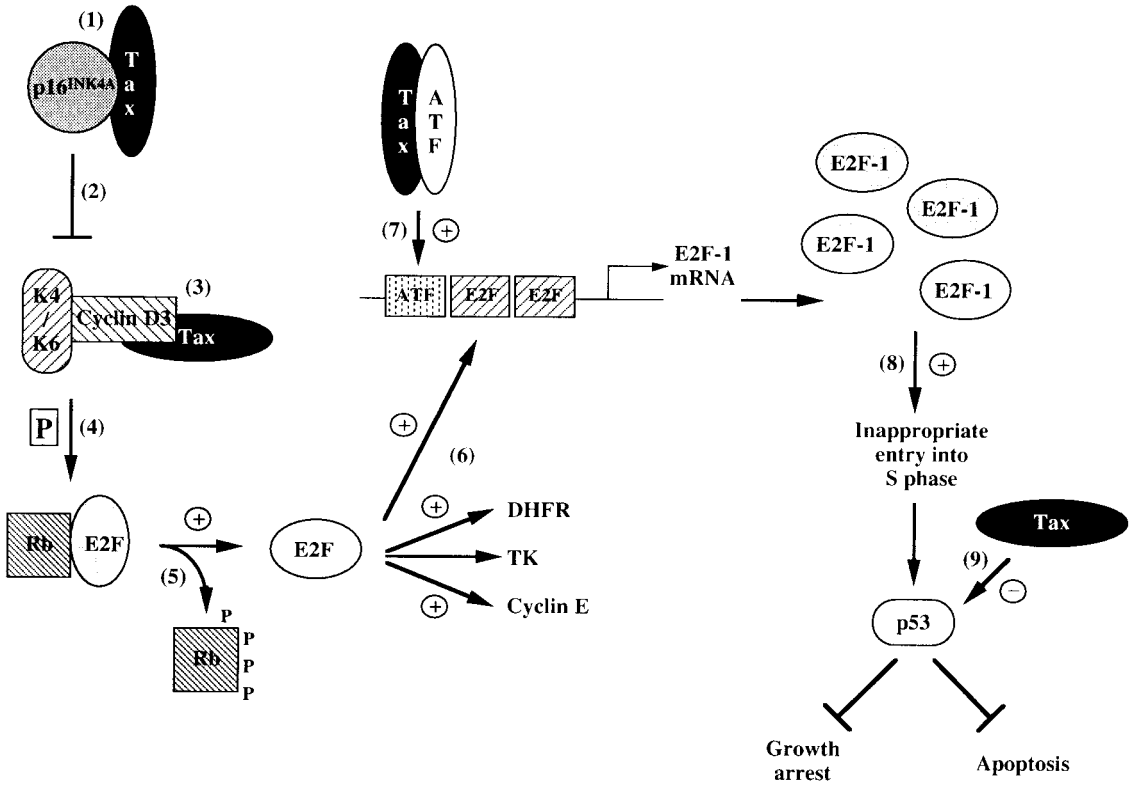

FIG. 3. Schematic representation of the activities of Tax that control cell proliferation. Tax binds to $p16^{INK4A}$ (1) and blocks the inhibitory activity of $p16^{INK4A}$ (2). Tax also interacts with cyclin D3, stabilizing the CDK–cyclin D3 complex (3). These events stimulate the activity of CDK4 (K4) and CDK6 (K6), which phosphorylate Rb (4). Phosphorylation of Rb destabilizes the Rb–E2F association (5) and stimulates expression of genes controlled by E2F (6). These genes code for proteins required for G_1- to S-phase transition such as dihydrofolate reductase (DHFR), thymidine kinase (TK), cyclin E, and E2F-1. The transcription of the last protein is also stimulated by Tax through the ATF/CREB pathway (7). Overexpression of E2F-1 promotes inappropriate entry into S phase (8), but Tax prevents p53-dependent induction of apoptosis or growth arrest by inhibiting p53 activity (9). The steps of the cell cycle blocked by Tax are shown by (⊣). The phosphorylation step is indicated by (Ⓟ). Stimulating and inhibitory effects are shown by (⊕) and (⊖).

that induces apoptosis upon binding to Fas ligand, FasL (Ashkenazi and Dixit, 1998). Tax has been shown to upregulate the *FasL* gene and thus to induce apoptosis (Chen *et al.,* 1997; Rivera *et al.,* 1998). However, the partial blockade of apoptosis by antibodies specific for interaction of FasL with its receptor indicates that the binding to FasL is not the major pathway of Tax-mediated apoptosis (Chlichlia *et al.,* 1997). On the other hand, the Tax effect was completely blocked by zVAD-fmk, a specific inhibitor of ICE (interleukin-1β-converting enzyme)-like proteases, suggesting that the apoptotic action of Tax requires ICE protease function (Chlichlia *et al.,* 1997). Thus it appears that Tax can activate both pro- and anti-apoptotic pathways, with the latter pathway being dominant in immortalized T cells. Consistent with this idea, oncogenic Ras has been shown to initiate a p53-independent apoptotic response that is suppressed through the activation of NF-κB (Mayo *et al.,* 1997). The stimulation of the NF-κB pathway by Tax could also be involved in a similar mechanism. Recent results obtained with CTLL-2, a mouse T-cell line, confirm this hypothesis. CTLL-2 cell lines expressing wild-type Tax or a Tax mutant, which is still an activator of NF-κB, are resistant to apoptosis induced in the absence of IL-2 (Iwanaga *et al.,* 1999). On the other hand, a Tax mutant, which is active for ATF/CREB but inactive for NF-κB, enhances apoptosis in the absence as well as in the presence of IL-2.

DNA oncoviruses encode proteins that inactivate Rb and p53, both proteins inhibiting progression to the S phase of the cell cycle. For instance, the adenovirus E1A stimulates the G_1- to S-phase transition by inactivating Rb. The inappropriate entry into S phase activates p53, causing growth arrest or apoptosis. However, two E1B proteins, p19 and p55, inhibit p53-mediated apoptosis. E1B 19K acts as a functional homolog of Bcl-2, whereas E1B 55K binds to p53 and inhibits its transcriptional activity (Evan and Littlewood, 1998; Linke, 1998). HTLV-1 has also developed its own strategy to counteract Rb and p53 functions (Fig. 3). HTLV-1 can stimulate the hyperphosphorylation of Rb by two independent mechanisms, both controlled by Tax. Tax can, at first, interact with $p16^{INK4A}$ to block its inhibitory activity and, second, stimulate CDK4 and CDK6 activities, perhaps by stabilizing the cyclin–CDK complexes as suggested for cyclin

D3 (Neuveut *et al.,* 1998). Moreover, Tax, through the ATF/CREB pathway, can activate the transcription of the gene encoding E2F-1. All these events favor overexpression of E2F-1, which then induces a p53-dependent apoptosis (Qin *et al.,* 1994; Wu and Levine, 1994). However, Tax is also able to inactivate p53 by a mechanism that, at present, remains unknown. It is noteworthy that HTLV-1 codes for only one viral protein that inhibits Rb and p53, whereas adenovirus encodes three different proteins that have the same effect. It is likely that the explanation resides in the difference in genome size between the two viruses. Since HTLV-1 has a genome that is much smaller than that of adenovirus, it is probably an advantage for the virus to code for a multifunctional protein such as Tax. However, the existence of other viral proteins encoded by the 3′ end of the HTLV-1 genome has been described (Koralnik *et al.,* 1992), and we cannot exclude the possibility that among these proteins one could also be involved in cell proliferation either directly or indirectly. For instance, HTLV-1 $p12^{I}$ is able to cooperate with the bovine papillomavirus E5 oncoprotein to transform mouse cells (Franchini *et al.,* 1993).

Recent results suggest also a dysregulation by Tax of the G_2–M checkpoint of the cell cycle. By interacting with protein MAD1 (mitotic arrest-defective) which is involved in the G_2–M transition control, Tax would inhibit MAD1 function (Jin *et al.,* 1998). This observation could explain the karyotypic abnormalities present in ATL cells. Thus, not only does Tax force infected cells into S phase but Tax could also dysregulate the G_2–M transition. The succession of both events could contribute to the transformation of T cells by HTLV-1.

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