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MINIREVIEW

and the NF-κB/IκB Transcription Complex

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The human T cell lymphotropic virus (HTLV-1) is the etiologic agent of adult T cell leukemia (ATL), an aggressive and fatal leukemia of CD4 T lymphocytes that generally occurs in late adulthood (Poiez *et al.,* 1980; Yoshida *et al.,* 1982). HTLV-1 infection is also associated with a neurological demyelinating disease known as tropical spastic paraparesis. ATL is geographically localized in regions of the world where HTLV-1 infection is endemic, notably southern Japan, sub-Saharan Africa, the Caribbean basin, and parts of the southwestern United States (Sodroski, 1992). Based on the phenotype of ATL cells *in vivo* and T cell lines transformed *in vitro* by HTLV-1, infection of $CD4^+$ T cells may initiate a multistep oncogenic process, characterized by an early polyclonal proliferation of T cells involving high levels of IL-2 receptor expression, increased production of interleukin-2, and dysregulation of growth factor production. Over a time course that encompasses decades *in vivo,* a growth factor independent, monoclonal population of leukemic T cells emerges.

The HTLV-1 viral genome is 9 kb in length and contains, in addition to the structural *gag, pol,* and *env* genes, an open reading frame in the 3′ end of the viral genome which encodes three viral regulatory proteins — Tax (p40X), Rex (p27X), and p21 (Seiki *et al.,* 1983). The oncogenic potential of HTLV-1 resides in the 40-kDa viral Tax protein which has been characterized as a positive regulator of viral gene transcription, acting via 21-bp repeats within the long terminal repeat (LTR). Tax also transactivates a number of cellular genes which likely play a role in early leukemogenic events, including IL-2, IL-2R-α, GM-CSF, c-fos, vimentin, IL-6 (reviewed in Yoshida, 1993). Since Tax is not a DNA binding protein per se, Tax transactivation occurs indirectly via physical interaction between Tax and host proteins that are targets for transactivation including: $p67^{SRF}$, CREB, Ets-1, TBP, ATF, and NF-κB/Rel proteins (Suzuki *et al.,* 1993a,b; Zhao and Giam, 1991; Franklin *et al.,* 1993; Wagner and Green, 1993; Fujii *et al.,* 1992; Caron *et al.,* 1993; Beraud *et al.,* 1994; Hirai *et al.,* 1992). Recent series of publications have investigated the associations between HTLV-1 Tax and the NF-κB/Rel proto-oncogene family. The purpose of this minireview is to provide an overview of these studies and to evaluate the role of Tax–NF-κB interactions in the initial events associated with T cell transformation by HTLV-1.

STRUCTURE–FUNCTION OF THE Tax PROTEIN

Tax is a 353-amino-acid (aa) protein that localizes to the nuclei of HTLV-1-infected cells, mediated via an N-terminal, atypical nuclear localization sequence (Sodroski, 1992). Mutagenesis studies (Smith and Greene, 1990; Semmes and Jeang, 1992) demonstrated that distinct amino acid residues of Tax were required for transactivation of the CREB and NF-κB pathways (Fig. 1). Mutation of a region located between amino acids 315 and 325 abrogated CREB-mediated Tax transactivation without affecting NF-κB-mediated transactivation. Conversely, scattered mutations in Tax between amino acids 113 and 258 resulted in intact CREB but deficient NF-κB-mediated transactivation, demonstrating that Tax transactivation occurred via distinct regions of the viral protein that targeted specific transcription factors (Smith and Greene, 1990; Semmes and Jeang, 1992). The transactivation phenotypes of the Tax mutants such as M22 ($CREB^+$/NF-$κB^-$) and M47($CREB^-$/NF-$κB^+$) have served as valuable reagents in the dissection of Tax–host transcription factor interactions (Smith and Greene, 1990).

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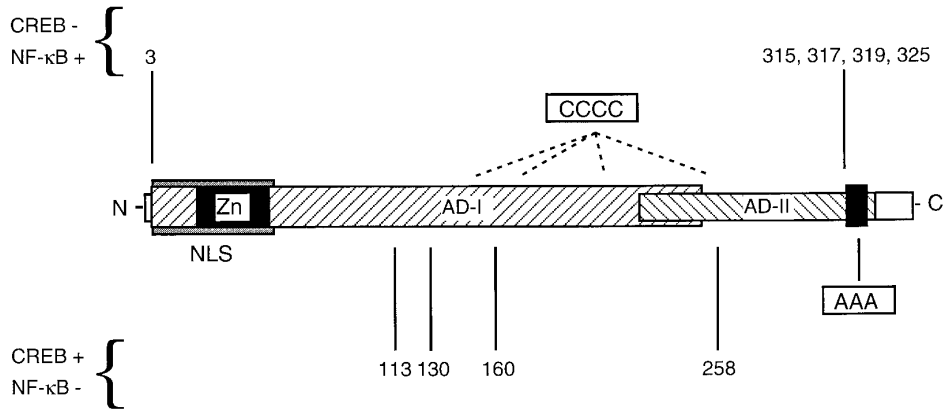

FIG. 1. Schematic organization of the HTLV-1 Tax protein. The structure of the 353-amino-acid (aa) Tax protein is illustrated schematically. The N-terminal gray box indicates the nuclear localization signal, NLS. The cysteine-rich zinc binding domain is represented by a black box marked "Zn" (aa 22–53). The white box denoted "CCCC" indicates the second cysteine-rich region involved in multimer formation (at aa positions 153, 174, 212, and 261). The C-terminal black box labeled "AAA" denotes the acidic aa stretch (aa 323–332). The transcriptional activation domains (AD-I, aa 2–255, and AD-II, aa 227–337) are represented by hatched boxes. The numbers above and below the diagram indicate the aa positions of mutations that abrogate CREB-mediated (above) or NF-κB-mediated (below) transactivation (Sodroski, 1992; Smith and Greene, 1990; Semmes and Jeang, 1992, 1995; Tsuchiya *et al.,* 1994).

Mutational analysis, however, failed to define discrete functional domains mediating Tax transactivation, indicating that about 95% of the protein was indispensible for this activity. Chimeric proteins fusing Tax to a Gal4 DNA binding domain identified a transactivation function of Tax that was separable into two overlapping stretches, AD-I (aa 2–255) and AD-II (aa 227–337), both of which were required for the formation of a functional activation domain (Tsuchiya *et al.,* 1994). Using a similar assay (a Gal4-Tax fusion protein and a responsive promoter containing Gal4 consensus binding sites), Semmes and Jeang determined that activation by the Gal4-Tax fusion was "squelched" by coexpression of wild-type Tax protein *in trans.* Tax mutants containing changes in amino acids 289 to 322 failed to inhibit Gal4-Tax activation (Semmes and Jeang, 1995). Furthermore, the 289- to 322-aa domain of Tax fused to Gal4 was able to activate a core promoter, indicating that this region defines a modular activation domain that contacts an essential transcription factor (Semmes and Jeang, 1995).

ONCOGENICITY BY Tax

The process of HTLV-1 infection *in vitro* — in terms of T cell proliferation, IL-2 dependency, IL-2R expression, and proviral integration — is reminiscent of ATL development. Support for a role of Tax in leukemogenesis derived initially from the analysis of leukemic cells from ATL patients that contained defective HTLV-1 proviral genomes which preferentially retained the 3′ ORF encoding Tax (Yoshida, 1993). Subsequent studies *in vitro* demonstrated that overexpression of Tax could immortalize primary T cells, although the cells remain IL-2 dependent (Grassmann *et al.,* 1994). Introduction of Tax into primary rat embryo fibroblasts immortalized cells or postponed senescence and, in cooperation with the *ras* oncogene, transformed primary fibroblasts such that they were tumorigenic in nude mice (Pozzatti *et al.,* 1990; Tanaka *et al.,* 1990). These Tax-transformed cells spontaneously reverted to a normal phenotype when the *tax* gene was lost (Yamaoka *et al.,* 1992). Importantly, HTLV-1-mediated transformation both *in vivo* and *in vitro* induced an IL-2/IL-2R autocrine loop that stimulated T cell proliferation.

Transgenic mice expressing the Tax protein presented heterologous phenotypes ranging from no malignancies to thymic aplasia, neurofibromas, exocrinopathies, arthropathies, and sarcomas (Nerenberg *et al.,* 1987). Interestingly, irrespective of transformation phenotype, cells derived from Tax transgenic animals produced elevated amounts of GM-CSF and IL-2 receptors (Green *et al.,* 1989). Although the development of ATL-like malignancy was not detected in Tax transgenic mice, double transgenic animals that carry the HTLV-1 LTR-driven c-*myc* and Ig promoter/enhancer-driven *tax* genes were found to develop central nervous system tumors and $CD4^+$ T cell lymphomas, similar to those occurring in HTLV-1-infected individuals (Benvenisty *et al.,* 1992). Transgenic mice with expression of Tax restricted to mature T lymphocytes by using the human granzyme B promoter developed large granular lymphocytic leukemia, demonstrating that Tax expression in the lymphocyte compartment was sufficient for the leukemia development (Grossman *et al.,* 1995).

Tax-MEDIATED TRANSACTIVATION OF CELLULAR GENES

In addition to IL-2 and IL-2Rα genes, Tax also transactivated many other genes involved in cell activation,

proliferation, and differentiation: cytokines (IL-3, IL-4, TNFα and β, GM-CSF, TGFβ, IFNγ, NGF, proenkephalin, parathyroid hormone-related protein), a cell surface receptor (MHC class I), a cytoskeleton protein (vimentin), several members of various transcription factor families (fos/jun, NF-κB/Rel, Egr/Krox, c-*myc*), viral enhancers (HIV, CMV), and a tyrosine kinase (c-*lyn*) (Yoshida, 1993). Interestingly, Tax repressed the human β-polymerase gene, the product of which is involved in DNA repair (Jeang *et al.,* 1990). These observations indicated that the HTLV-1 transformation potential resided in the ability of Tax to transactivate expression of cellular genes implicated in cell growth regulation.

NF-κB PROTEINS ARE TARGETS FOR Tax TRANSACTIVATION

One of the important targets of interaction for Tax protein is the NF-κB/IκB transcription complex. NF-κB DNA binding activity is constitutively elevated in HTLV-1-infected cells and is thought to contribute to the transformed phenotype (Yoshida, 1993). Significantly, inhibition of NF-κB activity by antisense RNA in cells derived from Tax transgenic animals results in loss of the transformed phenotype (Kitajima *et al.,* 1992).

The NF-κB/Rel transcription factors are a family of dimer-forming proteins that bind to the consensus DNA sequence 5′-GGGANNYYCC-3′, found in the promoter regions of cellular genes implicated in immunoregulatory and inflammatory processes, including cytokines, cell surface receptors, adhesion molecules, and acute phase proteins (Grimm and Baeuerle, 1993; Baeuerle and Henkel, 1994). Structurally, all DNA binding members of the family (NF-κB1 p50, NF-κB2 p52, RelA (p65), c-Rel, and RelB) share an amino-terminal *rel* homology domain involved in DNA binding, protein dimerization, and nuclear translocation (Grimm and Baeuerle, 1993; Baeuerle and Henkel, 1994). Homo- and heterodimer formations among the DNA binding members of the NF-κB family permit a range of differential transcriptional activities that may stimulate or inhibit gene expression (Perkins *et al.,* 1992; Lin *et al.,* 1995a).

NF-κB/Rel proteins exist in the cytoplasm coupled to inhibitory molecules, collectively termed IκB, that are responsible for cytoplasmic retention of NF-κB. IκB also constitutes a family of proteins including IκBα, IκBβ, IκBγ, bcl-3, p105, and p100, characterized by the presence of five to seven repeats of a 33-amino-acid ankyrin motif (Beg and Baldwin, 1993; Gilmore and Morin, 1993). NF-κB1 p105 and NF-κB2 p100 are non-DNA binding precursors of p50 and p52, respectively, that are proteolytically cleaved to generate the DNA binding subunits. The ankyrin repeat domains are located in the carboxyl-terminal half of the molecule and confer to the precursors IκB-like properties. IκBα specifically binds to and masks the nuclear localization signal of NF-κB/Rel proteins, thereby preventing nuclear translocation (Beg and Baldwin, 1993; Beg *et al.,* 1992). Cellular stimulation by multiple inducers (cytokines, virus infection, mitogens, radical oxygen intermediates) leads to the phosphorylation and subsequent degradation of IκBα. IκBα loss permits NF-κB/Rel dimer translocation to the nucleus and target gene activation (Beg and Baldwin, 1993; Gilmore and Morin, 1993). Degradation and resynthesis of IκBα appear to be general mechanisms determining the rapid but transient activation of gene activity by NF-κB (Beg *et al.,* 1993; Sun *et al.,* 1993; Brown *et al.,* 1993). Activated p65 and c-Rel proteins in turn induce *de novo* IκBα RNA expression, thus completing an autoregulatory loop that ultimately restores the cytoplasmic pool of latent NF-κB/IκB (Sun *et al.,* 1993; Brown *et al.,* 1993; Beg *et al.,* 1993; Le Bail *et al.,* 1993). NF-κB/Rel activity is also regulated at the transcriptional level, conferred by the presence of NF-κB sites in the promoters of NF-κB1 (p105 and p50), c-*rel,* MAD-3(IκBα), and NF-κB2 genes (Hannink and Temin, 1990; Le Bail *et al.,* 1993; Duckett *et al.,* 1993; Sun *et al.,* 1994a).

Changes in the balance of NF-κB subunits available in a particular cell may alter the pattern of gene expression and, by implication, contribute to oncogenic transformation. Several lines of evidence have demonstrated the involvement of NF-κB/*rel* in leukemogenic transformation: (1) v-*rel* is the oncogenic derivative of c-*rel* and is found in the virulent avian reticuloendotheliosis virus (REV-T). REV-T transforms and immortalizes avian T and B lymphocytes, as well as monocytes (Bose, 1992). v-*rel* has been shown to bind to NF-κB sites and inhibit the ability of NF-κB to stimulate target genes. Current evidence indicates that v-*rel* represents a dominant negative repressor that functions by overcoming the action of c-*rel,* its normal cellular counterpart (Ballard *et al.,* 1990). (2) Chromosomal translocations have been identified in human B cell lymphoma and chronic lymphocytic leukemia (CLL) that alter the structure and/or function of NF-κB2 (originally identified as lyt-10), in the t(10;14) chromosomal translocation (Schmid *et al.,* 1991; Neri *et al.,* 1991), c-Rel [the t(2;2) translocation in the RC-K8 lymphoma line (Lu *et al.,* 1991)], and the IκB molecule *bcl*3 [the t(14;19) translocation in CLL (Ohno *et al.,* 1990)].

Overexpression of IκBα antisense RNA but not IκBγ antisense RNA decreased the steady-state levels of IκBα, altered NF-κB DNA binding and gene activity, and induced malignant transformation as measured by saturation density, growth in soft agar, and tumorigenicity in nude mice. In contrast, overexpression of IκBα resulted in decreased saturation density, a flattened cellular morphology, and decreased NF-κB-dependent reporter gene activity. These results indicated that overexpression of an IκBα antisense RNA may disrupt the NF-κB/IκB autoregulatory loop, leading to cellular transformation, and

raise the possibility that IκBα may represent a potential growth suppressor activity (Beauparlant *et al.,* 1994).

Tax-MEDIATED PHOSPHORYLATION AND DEGRADATION OF IκBα

Tax-induced expression of NF-κB DNA binding activity in the nucleus of HTLV-1-infected or Tax-expressing human T cells is associated with increased phosphorylation and rapid proteolytic degradation of IκBα (Kanno *et al.,* 1994a; Sun *et al.,* 1994a; Lacoste *et al.,* 1995). The phosphorylated form of IκBα remains complexed with NF-κB/Rel dimers in the cytoplasm and is subjected to rapid proteasome-mediated degradation while in association with NF-κB (Finco *et al.,* 1994; Traenckner *et al.,* 1994; Lin *et al.,* 1995b; DiDonato *et al.,* 1995; Alkalay *et al.,* 1995).

The kinetics of Tax-mediated induction of NF-κB DNA binding activity were analyzed using an inducible Tax expression system, regulated in Jurkat T cells (JPX-9 cells) by the heavy-metal responsive metallothionein promoter. The initial NF-κB binding activity observed after Tax induction by zinc was composed predominantly of p50/p65 heterodimers, activated by a posttranslational mechanism involving a Tax-induced proteolytic degradation of IκBα (Kanno *et al.,* 1994a). At later times after Tax activation, NF-κB complexes containing c-Rel and NF-κB2 p52 accumulated, in part due to a transcriptional upregulation of c-Rel and NF-κB2 expression (Kanno *et al.,* 1994a). Upon induction of Tax expression, *mad*-3 gene expression was also increased. Although the steady-state levels of IκBα were unaffected by Tax expression in JPX-9 cells, IκBα turnover was increased significantly in cells expressing a wild-type Tax but not a mutant Tax protein (Smith and Greene, 1990). The positive autoregulation of c-Rel and NF-κB2 (Lanoix *et al.,* 1994; Li *et al.,* 1993; Sun *et al.,* 1994a), together with Tax-induced degradation of IκBα, alters the intracellular accumulation of NF-κB heterodimers and may continuously weaken IκBα inhibition of DNA binding activity.

Constitutive phosphorylation and increased degradation of IκBα were also detected in other HTLV-1-infected and Tax-expressing T cell models (Sun *et al.,* 1994b; Lacoste *et al.,* 1995). In general, an inverse correlation between Tax protein expression and steady-state levels of IκBα was observed in these studies; i.e., higher levels of Tax in HTLV-1-infected cells (MT-2 and C8166-45) resulted lower levels of IκBα, higher levels of phosphorylated IκBα, and faster IκBα turnover. Sun *et al.* demonstrated that Tax expression induced constitutive phosphorylation and rapid turnover of IκBα, with a $T_{1/2}$ <5 min in C8166-45 cells (Sun *et al.,* 1994a). This turnover rate differed significantly from that obtained by Lacoste *et al.* ($T_{1/2}$ ~40 min), but the discrepancy may be explained by the fact that Sun *et al.* measured turnover of the phosphorylated pool of IκBα, while degradation of the entire IκBα pool (both unphosphorylated and phosphorylated forms) was measured by Lacoste *et al.* IκBα mRNA transcript levels were also increased 7- to 20-fold in Tax-expressing cells, probably as a consequence of constitutive NF-κB binding activity and subsequent induction of the IκBα gene. Together these results support a model in which a HTLV-1 Tax-mediated phosphorylation of IκBα targets IκBα for degradation through the ubiquitin–proteasome pathway (Palombella *et al.,* 1994). Disruption of the NF-κB/IκB autoregulatory pathway results in constitutive NF-κB DNA binding activity that may promote aberrant NF-κB-dependent gene expression in T cells.

Experiments by Munoz *et al.* are at odds with the above results (Munoz *et al.,* 1994). Using murine 70Z/3 B cells and recombinant Tax protein uptake from the medium as inducer, Tax induced NF-κB translocation to the nucleus but did not induce IκBα degradation. Addition of cycloheximide indicated that the half-life of IκBα was reduced by Tax addition, but this effect was also induced by a Tax mutant that was unable to activate NF-κB. The authors concluded that the release of NF-κB stimulated by Tax represented the pool of NF-κB retained in the cytoplasm by p105 and/or p100 and that Tax did not induce IκBα degradation. The discrepancies between these results and the previous experiments (Kanno *et al.,* 1994a; Sun *et al.,* 1994a; Lacoste *et al.,* 1995) may be due to differences in the cell models used — murine B cells versus human T cells and/or the method of introducing Tax — direct uptake of protein versus DNA-mediated coexpression or analysis of HTLV-1-infected cells. These factors may significantly affect the interpretation of the results. Recent studies described below (Brockman *et al.,* 1995) also support a critical role for Tax in IκBα phosphorylation and degradation.

Mutational analysis of IκBα demonstrated that mutations at serine 32 or serine 36 of IκBα generated a molecule that did not undergo signal-induced phosphorylation or degradation, whereas mutation of other potential phosphoacceptor Ser, Thr, and Tyr residues in the NH_2-terminal 89 residues of IκBα did not block signal-induced phosphorylation or degradation of the protein (Brown *et al.,* 1995). Proteolysis of IκBα required additional COOH-terminal PEST sequences, a region rich in Pro Glu/Asp Ser and Thr residues often found in proteins that turn over rapidly, since a mutant lacking the 41 C-terminal residues was not proteolyzed in response to inductive signals. These studies demonstrated that signal-induced phosphorylation of IκBα at Ser 32 and/or Ser 36 was necessary for proteolysis of the inhibitor and for activation of NF-κB (Brown *et al.,* 1995). Phosphorylation of IκBα at Ser 32 and Ser 36 targeted the protein for ubiquitination *in vivo* and *in vitro,* whereas mutations that abolished phosphorylation and proteasome-mediated degra-

dation of IκBα also prevented ubiquitination *in vitro.* Thus the process of ubiquitination provides a mechanistic link between phosphorylation and degradation of IκBα (Chen *et al.,* 1995).

Removal of the N-terminal 36 amino acids of IκBα or specific point mutation of Ser-32 or Ser-36 blocked phosphorylation, degradative loss, and functional release of IκBα from NF-κB as a consequence of Tax overexpression (Brockman *et al.,* 1995). These NH_2-terminal alterations created transdominant repressors that escaped from Tax-induced turnover and inhibited NF-κB activation. Introduction of a phosphoserine mimetic at aa 32 and 36 corrected the IκBα defect, indicating a causal relationship between phosphorylation and proteolytic degradation of the inhibitor. It is unlikely that Tax directly destabilizes IκBα, since Tax–IκBα interactions have been observed *in vitro* only (Suzuki *et al.,* 1995). More likely, Tax associates with and/or activates a host kinase that phosphorylates IκBα, or Tax acts upstream of the kinase(s) responsible for inducible IκBα phosphorylation. These studies thus define a signal response domain in IκBα that regulates inducible degradation of IκBα in response to a diverse range of NF-κB activators that include tumor necrosis factor, T cell activation signals, and HTLV-1 Tax protein (Brown *et al.,* 1995; Brockman *et al.,* 1995; Chen *et al.,* 1995).

PHYSICAL INTERACTIONS BETWEEN Tax AND NF-κB SUBUNITS

A number of studies have recently focused on the physical associations between Tax and NF-κB/IκB subunits, often with seemingly contradictory results. Nonetheless, several points of consensus have now emerged. Using various assays, including coimmunoprecipitation of labeled proteins from HTLV-1-infected or Tax-expressing T cells, *in vitro* affinity chromatography, or cotransfection–immunofluorescence localization, it is clear that Tax interacts with high affinity with the NF-κB2 p100 precursor (Beraud *et al.,* 1994; Lanoix *et al.,* 1994; Sun *et al.,* 1994a; Kanno *et al.,* 1994b; Murakami *et al.,* 1995). Physical association is also detected with the NF-κB2 p52 product (Pepin *et al.,* 1994; Beraud *et al.,* 1994; Kanno *et al.,* 1994b), indicating that Tax binds to NF-κB2 via interaction with the Rel homology domain. The exact binding domains within either protein have yet to be determined.

One consequence of Tax–NF-κB2 interactions is a distinct intracellular pattern of sequestration of Tax protein; in cells overexpressing NF-κB2 p100, Tax is localized predominantly to the cytoplasm (Pepin *et al.,* 1994; Beraud *et al.,* 1994), whereas in cells overexpressing nuclear NF-κB2 p52, Tax is exclusively localized to the nuclei of transfected cells (Pepin *et al.,* 1994). Thus, the transcription modulatory influence of Tax may be significantly influenced by cytoplasmic–nuclear partitioning associated with the NF-κB proteins. The sequestration of Tax by p100 further implies a role for p100 in the maintenance of HTLV-1 latency observed in ATL (Beraud *et al.,* 1994).

Distinct multimeric complexes of NF-κB have also been described existing in the cytoplasm in association with the NF-κB2 p100 precursor (Sun *et al.,* 1994b; Kanno *et al.,* 1994b; Murakami *et al.,* 1995). The presence of these complexes in the cytoplasm is dependent on the ankyrin repeat domains located in the COOH-terminal half of the p100. Using metabolically labeled extracts from transfected cells and coimmunoprecipitation, the presence of a complex consisting of p100–p50–RelA was demonstrated (Kanno *et al.,* 1994b); interaction of p50–RelA heterodimers was dependent upon the COOH-terminal ankyrin repeat of p100. Tax expression produced an antagonistic effect on the cytoplasmic sequestration function of NF-κB2 p100, resulting in the release and nuclear translocation of p50–RelA dimers from the multimeric complex. A model to account for these observations proposes that Tax exerts its effects through physical association with the NH_2-terminal Rel homology domain of NF-κB2 p100, which in turn interferes with the inhibitory function of the C-terminal ankyrin domain (Kanno *et al.,* 1994b). There is, however, no evidence that the antagonistic effects of Tax on NF-κB2 p100 involve increased processing of the p100 precursor to its product p52 (Beraud *et al.,* 1994; Lanoix *et al.,* 1994). The presence of a cytoplasmic, p100-containing NF-κB reservoir that is targeted by Tax provides a distinct mechanism, in addition to Tax-induced IκBα degradation, by which Tax may augment the levels of nuclear NF-κB observed in HTLV-1-infected cells.

Previous studies have also demonstrated that Tax interacted physically with NF-κB1 p105, p50, and IκBγ (the 70-kDa protein derived from the carboxyl-terminal portion of p105), through both the Rel homology domain and the ankyrin repeat motif of the precursor molecule (Hirai *et al.,* 1992, 1994; Murakami *et al.,* 1995; Suzuki *et al.,* 1993b). These interactions stimulated nuclear translocation of NF-κB. The site of Tax binding within NF-κB1 p50 was localized between amino acids 140 and 341, the region of p50 required for dimerization and DNA binding (Suzuki *et al.,* 1995). The physical interaction between Tax and p105 has been controversial since some groups were unable to coimmunoprecipitate Tax and p105 complexes from HTLV-1-infected and Tax-transfected cells (Beraud *et al.,* 1994; Lanoix *et al.,* 1994; Sun *et al.,* 1994a). However, recent experiments demonstrated that both Tax–p100 and Tax–p105 complexes can be immunoprecipitated from cells, although the affinity of Tax for p100 was about fivefold higher than the affinity of Tax for p105 (Murakami *et al.,* 1995). Tax–IκBγ interactions were also documented in coexpression assays (Hirai *et al.,* 1994);

the biological significance of Tax–IκBγ interactions is unclear, since IκBγ has not been observed in T cells or any other human cells (Inoue *et al.,* 1992). More recently, interactions of Tax with RelA and c-Rel via the Rel homology domains have been described (Suzuki *et al.,* 1994). Some of the interactions between Tax and NF-κB members described above were detected under "forced" conditions — i.e., using *in vitro* affinity assays or evaluating extracts from cells engineered to overexpress individual or multiple proteins. It is therefore important to verify Tax–NF-κB interactions using several cell systems and relevant assays, particularly since the effects of Tax on NF-κB expression may be cell-line specific (Murakami *et al.,* 1995). These limitations notwithstanding, the evidence indicates that HTLV-1 Tax physically interacts with NF-κB2 with high affinity, and likely with other DNA binding members with lower affinity, via the Rel homology domain. The relative accumulation and cell-type-specific abundance of NF-κB proteins may also contribute to interactions *in vivo.*

CHANGES IN THE RELATIVE ABUNDANCE OF NF-κB SUBUNITS

Constitutive Tax expression leads to sustained nuclear expression of both c-Rel and p52 and dysregulated NF-κB DNA binding activity (Li *et al.,* 1993; Crenon *et al.,* 1993; Lanoix *et al.,* 1994; Sun *et al.,* 1994a,b; Kanno *et al.,* 1994a). Preferential c-Rel induction by HTLV-1 infection has been demonstrated at both the protein and the RNA levels, indicating that the effect is at least partially regulated at the transcriptional level (Li *et al.,* 1993; Crenon *et al.,* 1993). Furthermore, Tax transcriptionally activated the c-Rel promoter in coexpression experiments, and this activation may be controlled at least in part by RelA autoregulation (Li *et al.,* 1993; Sun *et al.,* 1994a). The NF-κB complex composition of HTLV-1-infected and Tax-expressing cells was different from that of Jurkat cells immediately following NF-κB stimulation (Lanoix *et al.,* 1994; Sun *et al.,* 1994b). HTLV-1-infected or Tax-expressing cell lines contained complexes composed of p50/c-rel and p52/c-rel heterodimers, whereas normal Jurkat cells following a 2-hr stimulation with PMA were composed exclusively of p50/RelA heterodimers (Lanoix *et al.,* 1994; Kanno *et al.,* 1994a).

As described above, kinetic analysis of NF-κB complexes in the Tax-inducible JPX-9 cell line demonstrated a direct correlation between Tax expression, degradation of IκBα, and nuclear recruitment of p50/p65 heterodimers to the nucleus (Kanno *et al.,* 1994a). At later times after activation, NF-κB complexes containing c-Rel and/or NF-κB2 p52 accumulated in the nucleus, in agreement with the high-level expression of c-Rel and p52 observed in HTLV-1-infected cells (Lanoix *et al.,* 1994; Sun *et al.,* 1994a; Kanno *et al.,* 1994a). Induction of NF-κB activity also stimulated c-Rel and NF-κB2 transcription, thus accounting for the sustained levels of c-Rel and p52 (Li *et al.,* 1993; Crenon *et al.,* 1993; Duckett *et al.,* 1993).

MULTIPLE MECHANISMS OF Tax-MEDIATED TRANSACTIVATION

The observation that Tax interacts physically with NFKB2(lyt-10) suggests that Tax may mediate its transforming potential at least in part via association with an NF-κB protein previously implicated in B and T cell leukemogenesis (Schmid *et al.,* 1991; Neri *et al.,* 1991; Fracchiolla *et al.,* 1993). In the B cell lymphoma associated chromosomal translocation t(10;14)(q24;q32), the amino-terminal DNA binding domain of NF-κB2(lyt-10) is juxtaposed to the immunoglobulin Cα locus, resulting in the generation of a NF-κB2-IgCα1 fusion protein. The t(10;14) chromosomal translocation produced a constitutively activated, DNA binding NF-κB2 molecule via loss of the ankyrin repeat-containing carboxy terminus and transcriptional deregulation of NF-κB2 caused by translocation into the transcriptionally active immunoglobulin locus (Schmid *et al.,* 1991). By analogy, HTLV-1, utilizing the Tax protein, may activate gene expression by interfacing with the NF-κB/*rel* signaling pathway at the level of the NF-κB2 protein.

It is not clear whether Tax remains associated with the NF-κB dimers translocating to the nucleus. However, other functions attributed to Tax suggest that it does. Tax enhanced the DNA binding activity of the ATF/CREB cellular proteins that bind to the HTLV-1 21-bp repeats (Giam and Xu, 1989). This activity was mediated through the DNA binding and dimerization domains of these proteins and did not require formation of stable Tax-containing complexes (Franklin *et al.,* 1993). Tax was also shown to increase the *in vitro* DNA binding activity of ATF/CREB proteins by stimulating dimerization and thus facilitating the DNA binding reaction (Wagner and Green, 1993; Baranger *et al.,* 1995). Enhancement of dimerization and DNA binding may allow dimers containing at least one strong transcriptional activation domain (e.g., RelA or c-Rel) to efficiently activate transcription, perhaps without further involvement of Tax. The capacity of Tax protein to increase the binding of cellular transcription factors to DNA via an increase in dimerization represents a unique mechanism by which Tax may mediate its effects on gene expression. The Tax transactivation function could play a direct role in stimulating transcription via association with subunits such as p52 or p50, which are strong DNA binding proteins that lack a transactivation function. The resulting complexes would combine both DNA binding and transactivation functions necessary to induce transcription (Yoshida, 1993).

The model emerging from these recent studies is that expression of the Tax protein in HTLV-1-infected cells

targets the NF-κB/IκB transcription complex, as well as other host signaling pathways, at multiple regulatory levels. Tax mediates the induction of IκBα phosphorylation and rapid degradation, the formation of complexes between Tax and NF-κB2, as well as other family members, and indirectly mediates the transcriptional activation of c-Rel, NF-κB2, and IκBα genes by an autoregulatory mechanism. These molecular events culminate in constitutive nuclear NF-κB DNA binding activity composed of multiple heterodimers of c-Rel, NF-κB2 p52, NF-κB1 p50, and RelA and transcriptional activation of NF-κB-regulated genes. Interestingly, induction of IL-2 and IL-2Rα expression establishes a positive autostimulatory loop that leads to the constitutive activation of the Jak-STAT pathway (Migone *et al.,* 1995). A polyclonal population of proliferating HTLV-1-infected T lymphocytes undergoes additional mutational events (Sakashita *et al.,* 1992) that, over a time period involving decades *in vivo,* favor the appearance of a leukemic population of T cells.

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