The pleiotropic cellular coactivator CREB binding protein (CBP) plays a critical role in supporting p53-dependent tumor suppressor functions. p53 has been shown to directly interact with a carboxyl-terminal region of CBP for recruitment of the coactivator to p53-responsive genes. In this report, we identify the KIX domain as a new p53 contact point on CBP. We show that both recombinant and endogenous forms of p53 specifically interact with KIX. We demonstrate that the activation domain of p53 participates in KIX binding and provide evidence showing that this interaction is critical for p53 transactivation function. The human T-cell leukemia virus, type I-encoded oncoprotein Tax is a well established repressor of p53 transcription function. Like p53, Tax also binds to KIX. The finding that both transcription factors bind to a common region of CBP suggests that coactivator competition may account for the observed repression. We demonstrate reciprocal repression between Tax and p53 in transient transfection assays, supporting the idea of intracellular coactivator competition. We biochemically confirm coactivator competition by directly showing that both transcription factors bind to KIX in a mutually exclusive fashion. These data provide molecular evidence for the observed intracellular competition and suggest that Tax inhibits p53 function by abrogating a novel p53-KIX interaction. Thus, Tax competition for the p53-KIX complex may be a pivotal event in the human T-cell leukemia virus, type I transformation pathway.

Adult T-cell leukemia is an aggressive and fatal hematological malignancy that is etiologically linked to infection with the human T-cell leukemia virus, type I (HTLV-I). Although there is a clear association between HTLV-I infection and adult T-cell leukemia, only a small percentage of infected individuals develop leukemia, and this generally follows a prolonged, asymptomatic latency period (reviewed in Ref. 1). The molecular mechanism of malignant transformation by the virus is poorly understood; however, expression of the virally encoded Tax protein appears to be a critical event in the leukemogenic pathway (2, 3).

Tax is a regulatory protein that is critical for high level HTLV-I transcription and thus is required for propagation of the virus. To activate viral gene expression, Tax participates in a series of protein-protein and protein-DNA interactions, forming a stable nucleoprotein complex on the HTLV-I promoter (4–12). Within this complex, Tax serves as a high affinity binding site for the recruitment of the pleiotropic cellular coactivator, CREB-binding protein (CBP) (13, 14). Once associated with the viral promoter, CBP is believed to remodel chromatin and/or facilitate communication with the basal transcription machinery. To recruit CBP, Tax has been shown to specifically bind to a region of CBP called the KIX domain (13, 14). This region of CBP also interacts with several other transcription factors, including Ser-133-phosphorylated CREB (Ref. 15 and reviewed in Ref. 16). Tethering of CBP to the HTLV-I transcriptional control region promotes the strong transcriptional activation associated with Tax (17).

In an effort to understand the role of Tax in leukemogenesis, several studies have examined the activity of the tumor suppressor protein p53 in HTLV-I-transformed T-cells (18–26). p53 is a transcription factor that induces cell cycle arrest or apoptosis in response to DNA damage, thus preventing the transmission of genetic mutations to the daughter cells (reviewed in Ref. 27). Loss of p53 activity has been identified in 60% of the human malignancies examined (28, 29), consistent with a role for p53 in genome surveillance and the suppression of oncogenic transformation. The tumor suppressor functions of p53 are strongly linked to its transcriptional activation properties. To stimulate transcription, the p53 activation domain binds to a poorly defined carboxyl-terminal region of CBP, tethering the coactivator to promoters of target genes (30–32). These observations suggest that CBP plays a critical role in supporting p53-dependent cell cycle arrest and apoptosis.

Although most human malignancies carry p53 mutations, the protein is generally present and wild type in HTLV-I-transformed cells (18–20). Paradoxically, these cells do not respond appropriately to a variety of stimuli that typically activate p53 (21–23). For example, γ-irradiation, which normally induces p53 transcriptional activity, has little or no effect on the expression of a panel of known p53-responsive genes in HTLV-I-transformed cell lines (24). These effects appeared to be due to Tax, as expression of the Tax protein increased the sensitivity of a mouse cell line to ionizing radiation-induced DNA damage and abrogated both p53-induced cell cycle arrest and apoptosis (25). Furthermore, Tax expression specifically inhibited p53 transcriptional activity, as measured by a p53-responsive reporter construct (20, 26). These data provide strong evidence suggesting that Tax inactivates p53, inhibiting the physiologically relevant transcription functions of this tumor suppressor protein (26). The inactivation of p53 by Tax...
likely represents a pivotal event in the transformation pathway. At present, the molecular basis for Tax inactivation of p53 transcription function is unknown.

Because both Tax and p53 utilize CBP to activate transcription, we considered direct coactivator competition as a conceivable mechanism for the observed Tax repression of p53 function in vivo. It is noteworthy that CBP protein is generally present at limiting concentrations within the cell nucleus, creating an environment of coactivator competition between transcription factors and providing an additional layer of regulated gene expression (33). Several recent studies suggest that a functional antagonism between transcription factors occurs as a consequence of direct competition for binding to common regions of CBP (34–40). Because p53 and Tax have previously been reported to bind to distinct regions of CBP, this mechanism initially appeared unlikely. However, in a recent report describing the solution structure of phosphorylated CREB bound to the KIX domain of CBP, the authors integrate several recent findings that may resolve this apparent paradox (41). In that study, specific amino acids within the kinase-inducible domain of CREB were shown to interact within a hydrophobic groove on the surface KIX. The authors noted that sequences within the activation domain of p53 share similarity with the kinase-inducible domain of CREB. Furthermore, they pointed out that the crystal structure of the mdm2-p53 complex shows that the activation domain of p53 binds to a hydrophobic groove on the surface of mdm2 (42). From these two seminal observations, Radhakrishnan et al. (41) predicted that p53 directly interacts with the hydrophobic groove on the surface of KIX. If the prediction is correct, it would provide strong support for direct coactivator competition between Tax and p53.

In this study, we examined the proposed interaction between p53 and the KIX domain of CBP. We show that both recombinant and endogenous forms of p53 specifically interact with KIX. The activation domain of p53 participates in KIX binding, and this interaction appears to be important for p53 transcription function. We extend these studies to show that Tax repression of p53-mediated transcription occurs through competition for the p53-KIX interaction. We also show that p53 represses Tax transcription function, as would be predicted from coactivator competition. Our evidence indicates that reciprocal repression between p53 and Tax arises directly from intracellular competition for the KIX domain of CBP. Finally, we show that Tax and p53 bind to KIX in a mutually exclusive fashion in vitro, providing a molecular basis for the intracellular competition. Together, these data suggest that Tax may inactivate p53 by specifically competing for the p53-KIX interaction. These observations may contribute key insight into the molecular events that lead to HTLV-I-associated leukemogenesis.

EXPERIMENTAL PROCEDURES

Cloning, Expression and Purification of Recombinant Proteins—GST-KIXp53–195, the GST-ΔKIX deletion mutants, and the GST-KIX point mutants previously have been described in detail (14, 43). GSTCH/1-KIX was made by PCR amplification of the CBP cDNA sequence corresponding to amino acids 302–683 (pCR/CRSV-CBP) (44) and insertion of the fragment into the BamHI site of pGex2T (Amersham Pharmacia Biotech). GST-CH/1 was made by digestion of the GST-CH/1-KIX PCR product with EcoRI, releasing a 471-base pair fragment corresponding to CBP amino acids 302–451. This fragment was inserted into the BamHI/EcoRI site of pGex2T. Both expression plasmids were transformed into SC51 cells (Stratagene), and the proteins were purified as described previously (43).

Three fusion proteins encompassing the carboxyl-terminal region of CBP were made by PCR amplification of the appropriate sequences from pCR/RSV-CBP (44). The PCR fragment from region 1, which carries sequences corresponding to amino acids 1514–1894, was inserted into the BamHI site of pGex2T. PCR fragments from both region 2, which carries sequences corresponding to amino acids 1894–2221, and region 3, which carries sequences corresponding to amino acids 2212–2441, were inserted into the EcoRI/BamHI site of pGex2T. The expression plasmids for regions 1 and 3 were transformed into SC51 cells, and region 2 was transformed into BL21(DE3) pLysS (45). Cell cultures were expanded and induced, and the GST proteins were purified by glutathione–agarose chromatography. Histidine-tagged p53 (HIS-p53) was cloned by PCR amplification of the wild type p53 cDNA (p53-H-19) (28). The PCR product was inserted into the EcoRI/BamHI site of pRSETA, transformed into BL21(DE3) pLysS, expanded, and induced as described previously (45). p53 was purified to greater than 90% homogeneity by Ni2+–NTA agarose chromatography (Qiagen). The purified protein was dialyzed against p53 dialysis buffer (20 mM Tris, pH 8.0, 0.5 mM EDTA, 0.1 mM KCl, 20 μg/ml glycerol, aliquoted, and stored at −70 °C. The histidine-tagged double point mutant of p53 (L22Q/W23S) was created as described above using the wild type His6-p53 and Stratagene’s Quick-Change site-directed mutagenesis kit. The DNA was transformed into BL21(DE3) pLysS, expanded, induced, and purified exactly as wild type His6-p53. Tax protein was expressed from the pTaxHis expression plasmid (46) and purified as described previously (14).

GST Pull-down Assays—All GST pull-down experiments were performed using 12.5 μl of glutathione–agarose beads equilibrated in 0.5× Superdex buffer (1× Superdex contains 25 mM Hepes, pH 7.9, 12.5 mM MgCl2, 10 μM ZnSO4, 150 mM KCl, 20% glycerol, 0.1% Nonidet P-40, 1 mM EDTA). The indicated amount of purified GST-CBP fusion protein was incubated with the beads for 1–2 h at 4 °C and for two times with 0.5× Superdex buffer. The indicated amount of the second protein was then added to the washed beads and incubated for 1–2 h at 4 °C. The beads were washed twice as before, and bound proteins were eluted with SDS sample dyes. Bound proteins were separated by electrophoresis on a 10% SDS gel, transferred to nitrocellulose, and probed with the appropriate antibody. The following antibodies were used in this report: anti-Tax antibody (epitope corresponding to the carboxy-terminal 13 amino acids), anti-p53 antibody (DO-1, Santa Cruz Biotechnology) (epitope corresponding to amino acids 11–25), anti-p53 antibody (Ab-1, Calbiochem) (epitope corresponding to amino acids 371–380), and anti-His antibody (H-15, Santa Cruz Biotechnology). The antibody inhibition pull-down experiments were performed as above, except that the indicated amount of p53 was preincubated with the indicated amount of either anti-p53 (DO-1), anti-p53 (Ab-1), or anti-epidermal growth factor receptor (Santa Cruz Biotechnology) (cell surface epitope) for 15 min at 4 °C prior to addition to either GST alone or GST-KIXp53–195.

Cell Culture, Transient Cotransfection Assays, and Mammalian Expression Plasmids—HTLV-I negative Jurkat T-cells were cultured in Iscove’s medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and penicillin-streptomycin. For transient cotransfection assays, cells were grown to a density of 106 cells/ml and transfected with LipofectAMINE (Life Technologies, Inc.) and a indicated amount of either anti-p53 (DO-1), anti-p53 (Ab-1), or anti-Tax (48) (48), cytomegalovirus-K88A Tax point mutant (49), and RSV-KIX (CBP amino acids 459–679) (14) have been previously described. The luciferase reporter plasmids pG13-Luc (50) and viral CRE-Luc (14) have also been described. The expression plasmid for the double point mutant of p53 (L22Q/W23S) was created using the wild type pC53-SN3 plasmid and Stratagene’s Quik-Change site-directed mutagenesis kit.

GST Pull-out Assays—HTLV-I-infected SLB1 human T-cells were grown to a density of 1 × 106 cells/ml in Iscove’s modified Dulbecco’s medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and penicillin-streptomycin. The nuclei were prepared using a Turner Designs model TD 20-ε luminometer.

Puriﬁcation of the GST plasmids for p53–SN3 (74) and type Tax (IE–Tax (48), cytomegalovirus-K88A Tax point mutant (49), and RSV-KIX (CBP amino acids 459–679) (14) have been previously described. The luciferase reporter plasmids pG13-Luc (50) and viral CRE-Luc (14) have also been described. The expression plasmid for the double point mutant of p53 (L22Q/W23S) was created using the wild type pC53-SN3 plasmid and Stratagene’s Quik-Change site-directed mutagenesis kit.
p53 Binds KIX in Vitro—Previous studies have shown that p53 physically interacts with a poorly defined carboxy-terminal region of CBP to activate transcription (30–32). We were interested in testing whether p53 may also interact with the carboxy-terminal region of CBP to activate transcription (30–32). We were interested in identifying specific amino acids in KIX that abolished interaction with p53. Two GST-KIXaa588–663 proteins (Fig. 1A), containing double point mutations at positions 603 and 659 or positions 600 and 633, abolished interaction with p53 (Fig. 1C, lanes 9 and 10). It is interesting to note that the pattern of p53 binding to the KIX deletion mutants and point mutants parallels the pattern of Tax binding to KIX (43); both serine 133-phosphorylated CREB and c-Jun recognize a slightly smaller region of KIX (43, 52, 54). In carrying out these studies, we also tested the binding of p53 to a larger region of KIX, GST-KIXaa451–719, and observed only a weak interaction (Fig. 1C, lane 3). We have previously observed that GST-KIXaa451–719 interacts weakly with other KIX-binding transcription factors, including c-Jun (54), suggesting that it forms an inappropriately folded structure that may prevent transcription factor access to the core structural domain of KIX. It is possible that the use of larger KIX molecules in previously published studies may have precluded detection of the p53-KIX interaction (30–32, 55).

The p53 Activation Domain Interacts with KIX—To establish the biological relevance of p53 binding to KIX, we were interested in identifying the domain of p53 involved in the interaction. It seemed plausible that the activation domain would play a role in coactivator recruitment. In fact, comparison of the activation domains of p53 and CREB suggested that this domain of p53 may directly bind the hydrophobic groove on the surface of KIX (41). We first tested whether an antibody directed against the p53 activation domain (ο-p53 aa 11–25) might block p53 binding to KIX in a GST pull-down experiment. In the same experiment, we also tested the effect of an antibody directed against the carboxyl terminus of p53 (ο-p53 aa 371–380) and an unrelated antibody (ο-epidermal growth factor receptor). Fig. 2A shows that the anti-p53 activation...
domain antibody strongly blocked p53 binding to KIX (lanes 4 and 5). Equal concentrations of the other two antibodies had no detectable effect on p53 binding to KIX (Fig. 2A, lanes 6–9). These results suggest that a region near or within the activation domain of p53 participates in KIX binding.

To determine whether specific activation domain amino acids are involved in the interaction, we introduced a double point mutation (L22Q/W23S) into the activation domain of p53 and tested the ability of the mutant protein to bind KIX. We selected these two amino acids in the p53 amino terminus because the negative effect of these mutations on p53 transcription function has been well characterized (56). Fig. 2B shows the results of a GST pull-down assay in which we tested the binding of purified wild type and mutant p53 proteins to several regions of CBP. Surprisingly, the double point mutation in the p53 activation domain completely abolished interaction with KIX (Fig. 2B, lanes 7 and 8). The activation domain mutant exhibited reduced binding to the two carboxy-terminal regions of CBP, consistent with previous studies (30) (Fig. 2B, lanes 9–12). Although the preparation of GST-KIXaa588–683 used in this experiment was less active for p53 binding, the interaction with mutant p53 protein was still significantly decreased (we typically observed significant variation in the activity of our GST-KIXaa588–683 preparations). As a negative control, we also tested mutant p53 binding to C/H1 (Fig. 1A). p53 amino acids 90–160 have previously been shown to interact with this region of CBP (53). No significant difference in the binding of wild type and mutant p53 protein to C/H1 was observed (Fig. 2B, lanes 5 and 6). Together, these data indicate that the activation domain of p53 interacts directly with KIX.

**Dominant Negative Repression of p53 Activity by KIX**—To determine the functional role of the p53-KIX interaction, we examined p53 transcription activity in transient transfection assays in the presence of increasing amounts of an expression plasmid for KIX (RSV-KIXaa459–679). Because KIX does not have intrinsic transcriptional coactivation properties, p53 binding to free KIX should block p53 interaction with endogenous CBP and therefore have a dominant negative effect on p53 transcriptional activity. Fig. 3 shows that titration of an expression plasmid for KIX produced a dose-dependent repression of p53-mediated transcription (lanes 3 and 4). These data suggest that p53 transcription function requires interaction with the KIX domain of CBP.

**Endogenous p53 Binds KIX**—We next tested whether endogenous p53 protein, present in a nuclear extract, interacts with KIX. For this experiment, we compared the binding of p53 to KIX, C/H1, and C/H1-KIX (Fig. 1A). To perform this experiment, GST-CBP fusion proteins were bound to glutathione-agarose beads and incubated with nuclear extracts from the human T-cell line SLB-1. Fig. 4 shows the result of the GST pull-out assay. As expected, the endogenous p53 protein interacted strongly with the KIX domain (GST-KIXaa588–683), but not with a carboxy-terminal deletion mutant of KIX (GST-KIXaa588–663) (Fig. 4, lanes 6 and 7). p53 exhibited the highest apparent affinity for GST-C/H1-KIXaa502–683 and also bound well to the C/H1 domain (Fig. 4, lanes 4 and 5). The strong binding of p53 to C/H1-KIX was not unexpected, as this larger CBP region encompasses two independent p53 recognition elements. Together, these studies provide evidence for a strong interaction between p53 and the KIX domain of CBP and support the idea that the KIX-p53 interaction is relevant for p53...
Reciprocal Repression between Tax and p53—Several previously published studies have reported Tax inactivation of p53 function; however, the molecular basis for the antagonism was not known (20–24, 26). Our experimental evidence establishing a p53-KIX interaction suggests that p53 and Tax may physically compete for the KIX domain of CBP in vivo. To directly test whether Tax and p53 compete for the KIX domain of CBP, we were first interested in confirming the previously published studies using our transient transfection assay system. The transcription function of p53 was measured using a reporter plasmid carrying 13 copies of the p53-response element driving expression of luciferase (pG13-luc). In HTLV-I-negative, p53-negative (Bax-luc), bax expression of luciferase (pG13-luc). In HTLV-I-negative, p53-negative (Bax-luc), bax was cotransfected with a constant amount of the Tax expression plasmid (IEX-Tax) (200 ng) and an increasing amount of the p53 expression plasmid (pC53-SN3) (50 and 100 ng), as indicated. The values shown are the average luminescence ± S.E. from two experiments performed in triplicate.

Reciprocal Repression between Tax and p53 Is Mediated through Competition for the KIX Domain of CBP—To more closely examine whether Tax repression of p53 transcriptional activity is mediated through CBP, we tested a Tax mutant that has previously been shown to be defective for interaction with KIX (49). This Tax mutant, carrying a single amino acid substitution at position 88 (K88A), is fully defective for repression of p53 mediated transcription (Fig. 5B, lanes 1 and 2). Cotransfection of increasing amounts of the p53 expression plasmid repressed Tax transactivation in a dose-dependent fashion (Fig. 5B, lanes 3 and 4). As shown in Fig. 5A (lanes 1 and 2), cotransfection of the p53 expression plasmid was not toxic to the cells. The reciprocal repression observed with these two transcription factors led us to further investigate whether the interference may be a result of competition for limiting amounts of CBP in the cell.

If repression of p53 by Tax occurs as a consequence of competition for CBP, then overexpression of p53 should similarly repress Tax function. To test this possibility, we performed the reciprocal experiment using a reporter plasmid carrying three copies of the Tax-responsive viral CRE (viral CRE-Luc) driving expression of the luciferase gene (14). Transfection of the Tax expression plasmid strongly activated transcription from the Tax-responsive promoter (Fig. 5B, lanes 1 and 2). Cotransfection assays were carried out in HTLV-I-negative Jurkat T-cells. The Tax-responsive viral CRE-Luc reporter plasmid (400 ng) was cotransfected with a constant amount of the Tax expression plasmid (IEX-Tax) (200 ng) and 50 ng of either the wild type or mutant p53 expression plasmid (pC53-SN3 wt and Δ22, 23, respectively) as indicated. The values shown are the average luminescence ± S.E. from two experiments performed in triplicate.

Reciprocal Repression between Tax and p53 Is Mediated through Competition for the KIX Domain of CBP—To more closely examine whether Tax repression of p53 transcriptional activity is mediated through CBP, we tested a Tax mutant that has previously been shown to be defective for interaction with KIX (49). This Tax mutant, carrying a single amino acid substitution at position 88 (K88A), is fully defective for Tax transactivation in vivo (49, 54). If Tax repression of p53 occurs through competition for the KIX domain of CBP, then the Tax K88A mutant should be unable to repress p53 function. Fig. 5C shows that Tax K88A is completely defective for p53 repression of Tax mediated transcription. Transient cotransfection assays were carried out in HTLV-I-negative Jurkat T-cells. The Tax-responsive viral CRE-Luc reporter plasmid (400 ng) was cotransfected with a constant amount of the Tax expression plasmid (IEX-Tax) (200 ng) and 50 ng of either the wild type or mutant p53 expression plasmid (pC53-SN3 wt and Δ22, 23, respectively) as indicated. The values shown are the average luminescence ± S.E. from two experiments performed in triplicate.
sion, as compared with wild type Tax (compare lanes 3 and 4). Both the mutant and wild type Tax proteins were expressed from the same promoter (cytomegalovirus), and Western blot analysis indicated that the mutant was properly expressed in the transfection assay (data not shown). To confirm that Tax K88A was defective for interaction with KIX, we tested the mutant protein in an in vitro binding assay. A purified GST fusion protein carrying the KIX domain (GST-C/H1-KIX<sub>aa302-683</sub> see Fig. 1A) was bound to glutathione-agarose beads and used in a GST pull-down assay with Tax. Equal amounts of purified wild type Tax and Tax K88A were tested for binding to KIX. Fig. 5D shows that, as compared with wild type Tax, K88A demonstrated significantly reduced interaction with KIX (compare lanes 3 and 4). The data obtained with Tax K88A are consistent with the hypothesis that Tax interaction with the KIX domain of CBP results in repression of p53 transcriptional activity. It is noteworthy that Tax K88A is fully defective for both activation and repression, strongly supporting the idea that a common mechanism is utilized in both pathways.

As shown in Fig. 2 above, the activation domain of p53 participates in direct binding to KIX, as a double point mutant in this region abolished the interaction. If p53 repression of Tax transcription function occurs through competition for KIX, then this p53 mutant should also be defective for Tax repression in vivo. To test this idea, we introduced the activation domain mutation into our mammalian p53 expression plasmid (data not shown). Fig. 5E shows that, as compared with wild type p53, the p53 activation domain mutant only modestly repressed Tax function (lanes 3 and 4). Both wild type and mutant p53 proteins were cloned in the same plasmid and expressed from the same promoter. Western blot analysis confirmed that both proteins were similarly expressed (data not shown). These data support our hypothesis that Tax and p53 compete for the KIX domain of CBP in vivo.

**Tax and p53 Compete for KIX in Vitro**—The observation that both p53 and Tax physically and functionally interact with KIX supports the idea that their binding is mutually exclusive, thus providing a molecular basis for Tax repression of p53 function in HTLV-I-transformed cells. To directly test this hypothesis, we examined whether increasing concentrations of Tax can displace p53 from KIX in vitro. For this experiment, glutathione-agarose beads were bound with GST-C/H1-KIX<sub>aa302-683</sub> and then incubated with purified, recombinant p53. We selected C/H1-KIX<sub>aa302-683</sub> (Fig. 5A) for this experiment, as the presence of the two adjacent p53 binding sites represents the p53-CBP interaction in a more physiologically relevant context. The C/H1-KIX fusion protein therefore enabled analysis of p53 binding to both sites in the presence of Tax. We have previously determined that Tax does not bind C/H1 and interacts only with KIX in this fusion protein (data not shown). Increasing amounts of Tax were included in the binding reactions containing p53, and the resulting protein-protein interactions were monitored by Western blot analysis. Fig. 6A shows that increasing amounts of Tax resulted in a reduction in p53 binding to C/H1-KIX<sub>aa302-683</sub>, with a concomitant increase in Tax binding (lanes 2–5). The observation that p53 binding is only partially inhibited by Tax suggests that Tax only competes for the p53-KIX interaction, leaving the p53-C/H1 interaction unperturbed. We next tested whether the Tax point mutant, Tax K88A, could effectively compete with p53 for KIX binding (Fig. 6B). We have previously shown that this Tax mutant is defective for binding to C/H1-KIX<sub>aa302-683</sub> (Fig. 5D). Fig. 6B shows that, as expected, increasing amounts of Tax K88A had no effect on p53 binding to C/H1-KIX<sub>aa302-683</sub> (lanes 2–5). We then performed a reciprocal competition experiment, binding Tax to GST-C/H1-KIX<sub>aa302-683</sub> fusion protein and adding increasing concentrations of p53. Titration of p53 into the binding reaction dramatically reduced Tax interaction with C/H1-KIX<sub>aa302-683</sub>, with a concomitant increase in p53 binding (Fig. 6C, lanes 3–6). Together, these data indicate that the binding of p53 and Tax to KIX is mutually exclusive and that elevated concentrations of either protein drives exclusive interaction with KIX.

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**Fig. 6. Tax and p53 binding to KIX is mutually exclusive.** A, Tax inhibits p53 binding to KIX. Purified p53 (25 pmol) was incubated with GST alone or GST-C/H1-KIX<sub>aa302-683</sub> (25 pmol) in the presence of the indicated amounts of purified Tax protein. p53 was detected by Western blot analysis, and bound p53 protein is indicated in the upper panel. The Western blot was reprobed using an anti-Tax antibody, and bound Tax is indicated in the lower panel. B, Tax K88A cannot inhibit p53 binding to KIX. Purified p53 (25 pmol) was incubated with GST alone or GST-C/H1-KIX<sub>aa302-683</sub> (25 pmol) in the presence of the indicated amounts of purified Tax K88A protein. p53 was detected by Western blot analysis and bound p53 protein is indicated in the upper panel. The Western blot was reprobed using an anti-p53 antibody, and bound p53 is indicated in the lower panel. Protein standards are indicated at the left. Four percent of output Tax protein is shown (lane 1, upper panel).
DISCUSSION

The tumor suppressor protein p53 has been previously reported to interact with C/H1 and a carboxyl-terminal domain of the coactivator CBP (30–32, 53). These interactions appear to mediate p53 degradation and transactivation, respectively. In this report, we show that p53 also interacts with the KIX domain of CBP and that this interaction is important for p53 transcription function. We provide evidence that strongly supports the idea that the p53-KIX interaction is physiologically relevant. For example, expression of the KIX domain in transient transfection assays inhibits p53 transactivation activity, suggesting that p53 interacts with KIX in vivo and that this interaction is critical for p53-mediated transcription. We further show that purified recombinant p53 and, perhaps more significantly, endogenous p53 protein recognizes a minimal region of KIX (aa 588–683). This region corresponds to a hydrophobic core domain of CBP that comprises a pleiotropic transcription factor binding site (41). The strength of the p53 interaction with KIX is comparable to that observed with the previously identified carboxyl-terminal region of CBP.

Significantly, the activation domain of p53 participates in the interaction with KIX. We show that an antibody directed against the activation domain of p53 disrupts the interaction with KIX, and a double point mutation in the activation domain of p53 abolishes interaction with KIX. These data indicate that the activation domain of p53 is directly involved in KIX binding and provide strong evidence for the functional relevance of the p53-KIX interaction. Although it is clear that p53 interacts with multiple regions of CBP, our data suggest that the KIX domain is an additional contact point on CBP critical for p53 transcription function in vivo.

Several previous studies have shown that the HTLV-I Tax protein inhibits many of the tumor suppressor functions of p53; however, the molecular mechanism of this inhibition has remained elusive (20–24, 26). The finding that p53 interacts with KIX suggests coactivator competition as a plausible mechanism for Tax inactivation of p53 transcription function. Consistent with coactivator competition, we also observed p53 repression of Tax transactivation, indicating a reciprocal antagonism. We have previously shown that, like p53, Tax also recognizes the minimal KIX amino acids located between 588 and 683 (43). Thus, it appears that both proteins recognize the same surface structure of KIX, which suggests that their binding is mutually exclusive. This was directly demonstrated using a competition binding assay in which we showed that Tax specifically competes for the p53-KIX interaction, and as expected, p53 competes for the Tax-KIX interaction. Because the p53-KIX interaction appears to play a role in p53 transcription function, these data provide a molecular basis for Tax repression of p53 in vivo. Together, these observations are consistent with several previous studies showing that in HTLV-I-infected and Tax-expressing cells, p53 is unable to activate target genes in response to various stimuli (21–24). These data support a model for Tax repression of p53 transcription function that is mediated through direct coactivator competition.

A previous report examining Tax repression of p53 (59) suggests that a possible mechanism for p53 inactivation arises from a Tax-dependent increase in amino-terminal p53 phosphorylation. However, this observation is in contrast with a subsequent finding by the same group showing that phosphorylation within this region of p53 increases p53 function and CBP recruitment (60). The precise biological consequences of Tax-dependent phosphorylation of p53 remain elusive. Our direct demonstration that p53 utilizes the KIX domain for coactivator recruitment, together with the evidence that Tax and p53 binding to KIX is mutually exclusive, provides a sound framework for the mechanism of p53 inactivation.

HTLV-I-associated adult T-cell leukemia is well characterized by chromosomal abnormalities and karyotypic abnormalities. However, studies of adult T-cell leukemia patients reveal that p53 mutations are relatively rare, as compared with other human malignancies. The development of such severe genetic mutations seems unlikely in the presence of functional p53. Because it is well established that p53 plays a critical tumor suppressor role, it seems likely that Tax inactivation of p53 transcription function may be pivotal in the leukemogenic process. We propose that the high affinity binding of Tax to the KIX domain of CBP results in at least the partial inactivation of p53 transcriptional activity, obviating the need for p53 mutation. Although Tax expression is generally low in an HTLV-I-infected T-cell (61), intermittent burst periods of Tax expression have been noted (62). During these burst periods, Tax may sequester CBP and derail p53 transcription function. This event would likely promote an environment in the infected T-cell that is tolerant of mutations and chromosomal instability. Thus, Tax competition for the p53-KIX interaction may be paramount in the HTLV-I transformation pathway.

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