Enhanced Activation of Tax-dependent Transcription of Human T-cell Leukemia Virus Type I (HTLV-I) Long Terminal Repeat by TORC3*

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Tax, a protein encoded by the env-pX gene of human T-cell leukemia virus type I (HTLV-I), interacts with various host cell transcription factors. Tax activates transcription from the long terminal repeat (LTR) of HTLV-I through association with cyclic AMP-responsive element-binding protein (CREB). Here, we present evidence that transducer of regulated cyclic AMP-response element-binding protein 3 (TORC3), a co-activator of CREB, is involved in Tax-induced transcriptional activation from the HTLV-I LTR. By using a luciferase assay system, we show that TORC3 alone can enhance transcription from the HTLV-I LTR, as well as from a cellular cyclic AMP-response element (CRE). Interestingly, we find that co-expression of TORC3 and Tax dramatically increased transcriptional activation at the HTLV-I LTR. We also show by glutathione S-transferase pull-down and co-immunoprecipitation experiments that TORC3 interacts with Tax. Using deletion mutant analysis, we identify the Tax interaction domain of TORC3 as a region spanning from amino acid 1 to 103, which contains a coiled-coil domain. These results provide important clues toward understanding the molecular mechanism of Tax-dependent transcriptional activation of the HTLV-I LTR.

HTLV-I is a retrovirus that causes adult T-cell leukemia (1) and has been implicated in the development of other diseases such as HTLV-I-associated myelopathy, tropical spastic paraparesis, and uveitis (2–5). The onset of adult T-cell leukemia is preceded by a long latent period, suggesting that the process of leukemogenesis depends on multiple steps involving various factors, including viral proteins (4, 6).

The viral oncoprotein Tax is encoded by the env-pX gene (8) and plays a principal role in the regulation of the proliferation and transformation of HTLV-I-infected T cells. Tax is a pleiotropic factor that interacts with many cellular proteins to regulate gene expression. For example, Tax regulates the NF-κB pathway using a variety of mechanisms. Tax is not only able to activate NF-κB signaling by binding to NF-κB, IκB, and IKKδ (9–11) but also activates transcription of cellular genes with NF-κB-binding sites in their promoter regions (12, 13), such as Bcl-xL, interleukin-2, and interleukin-2 receptor, to regulate proliferation and transformation of HTLV-I-infected cells (14, 15). Furthermore, Tax also directly binds to p16INK4a, a member of the INK4 family of cyclin-dependent kinase inhibitors, and suppresses its function (16–18), which leads to aberrant cell cycle progression.

In addition to regulating expression of host cell genes, Tax also regulates transcription of viral genes by interacting directly with the cellular transcriptional factor CREB to activate the HTLV-I-LTR (19). The Tax/CREB complex binds to the Tax-responsive element (TxRE) in the HTLV-I LTR, promoting viral transcription through the recruitment of co-activators such as CBP/p300 and p300/CREB-binding protein-associated factor, a process that does not require phosphorylation of CREB (20–22). The HTLV-I LTR is composed of three tandem repeats of TxRE, which contains a cyclic AMP-responsive element (CRE) flanked by GC-rich sequences. Although the Tax/CREB complex appears to be recruited predominantly to the TxRE, it is not recruited to the cellular CRE as to a functional complex, suggesting that the CRE-flanking sequence provides specificity for binding of various activation complexes (23). Indeed, protease sensitivity analysis demonstrated that the conformation of CREB differs according to the specific context of the CRE to which it is bound, indicating that the conformation of CREB is regulated by CRE-flanking sequences (24). Specifically, the weak association of CREB with the CRE sequence of TxRE appears to expose its Tax-binding domain, allowing easy access of Tax to CREB. Binding of Tax may then tighten the ternary complex composed of CREB, Tax, and TxRE. Analysis of this ternary complex suggested that the GC-rich sequence flanking the CRE element in TxRE is crucial for interaction with Tax since this sequence was protected by Tax in a manner that depended on the dose of CREB (25). Moreover, a drug that binds the minor groove of DNA was able to disrupt Tax binding to the CRE-flanking sequence, suggesting that the interaction of Tax with nucleotides in the minor groove of the flanking GC-rich sequence plays an important role in establishing the stable ternary complex. Interaction of protein factors with the flanking sequences in TxRE in HTLV-I-LTR in vivo has also been demonstrated by DNA protection assays (26).

However, these results do not fully explain the mechanism of Tax-dependent transcription of HTLV-I LTR. First of all, the
physiological relevance of the ternary complex reconstituted by in vitro synthesized CREB and Tax may be questioned since no evidence has been presented that this complex has any biological function. Furthermore, protein complexes interacting with the flanking sequence in vitro may contain additional factors besides CREB and Tax. For example, we previously identified a cellular protein, TAXREB03/SRL300, which interacts with the 3’-flanking region of TTxRE (27). This protein enhances Tax-dependent transcription and CREB binding to TTxRE in cooperation with Tax. Thus, the precise mechanism of how Tax activation of CREB leads to transcriptional activation of the LTR remains to be elucidated.

Recently, TORCs were identified as a family of CREB co-activators that bind to CREB and enhance CRE-mediated transcription in a phosphorylation-independent manner (28, 29). This transcriptional activation is facilitated by recruitment of the TATA-binding protein-associated factor 130 (TAF130) component of transcription factors IID (TFIID) to CREB. One member of the family, TORC3, is expressed at high levels in B and T lymphocytes (29). Based on these studies, we hypothesized that TORC3 might be involved in the transcriptional activation of the HTLV-I LTR by Tax. In this study, we examine the effect of TORC3 on Tax-mediated transcription regulated by the HTLV-I LTR. We find that TORC3 dramatically enhances Tax-mediated viral promoter activity. Furthermore, we show that TORC3 interacts with Tax directly via the N-terminal region of TORC3, which contains a coiled-coil domain.

MATERIALS AND METHODS

Plasmid Construction—cDNA encoding human TORC3 (accession number NM_022769) was obtained by RT-PCR from HEK-293T cells. The expression plasmid for TORC3 was constructed by inserting the full-length cDNA into EcoRI and XhoI sites of the epitope-tagged form of pcDNA3 (Invitrogen). The primers used for the PCR were 5’-aaagaatctATGGGCCGCTCGGCCGCTCG-3’ and 5’-aaatctagCTCAACTGCTGTCAGCATTGCAA-3’. Lowercase letters indicate a linker sequence containing EcoRI and Xhol sites, respectively. A reporter plasmid, pTxRE-luc, which drives luciferase expression under a promoter with five copies of the Tax-responsive element, TTxRE, was used. To construct plasmids expressing GST fusion TORC3 and its deletion mutants, pGST-TORC3, pGST-TORC3 (1–103 aa), pG-TORC3 (95–620 aa), pGST-TORC3 (298–620 aa), and the corresponding DNA fragments were amplified by PCR using their respective primers. The fragments were then subcloned into EcoRI and Xhol sites in pGEX-6P-1 (Amerham Biosciences). The sequences of the primers used for GST-TORC3, 5’-aaagaatctATGGGCCGCTCGGCCGCTCG-3’ and 5’-tttctagCTCAACTGCTGTCAGCATTGCAA-3’, for GST-TORC3 (1–103 aa), 5’-aaagaatctATGGGCCGCTCGGCCGCTCG-3’ and 5’-tttctagCTCAACTGCTGTCAGCATTGCAA-3’, and for GST-TORC3 (298–620 aa), 5’-aaatctagCTCAACTGCTGTCAGCATTGCAA-3’. The expression plasmid for GAL4 fusion Tax was constructed by inserting full-length Tax into BamHI and SalI sites in pGAL4-luc. Full-length Tax was amplified by PCR using 5’-aaagaatctATGGGCCGCTCGGCCGCTCG-3’ and 5’-tttctagCTCAACTGCTGTCAGCATTGCAA-3’ as primers and pcDNA3-Tax (30) as a template. Lowercase letters indicate a linker sequence containing EcoRI and Xhol sites, respectively.

Cell Culture—HEK-293T cells were maintained in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin and were cultured at 37 °C in a 5% CO2 humidified chamber.

Plasmid Transformation—HEK-293T cells were grown to 50% confluence in 60-mm dishes or 24-well dishes in Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum. Cells were transfected with expression vectors or containing empty vector constructs using FuGENE 6 (Roche Applied Science) according to the manufacturer’s instructions.

Antibodies—Rat anti-HA (3F10; Roche Applied Science) and mouse anti-Myc (9E10; Santa Cruz Biotechnology) antibodies were purchased commercially. A rabbit polyclonal antibody specific for Tax was raised against the bacterially expressed GST-Tax (233–353 aa) fusion protein. Horseradish peroxidase-linked goat antibodies to rat IgG were from Jackson ImmunoResearch Laboratories. Horseradish peroxidase-linked goat antibodies to mouse or rabbit IgG were from Amersham Biosciences.

GST Pull-down Assay—GST fusion proteins were expressed in Escherichia coli and isolated by an affinity column with glutathione-Sepharose-4B beads (Amersham Biosciences). GST fusion proteins bound to the resin were then incubated with [35S]methionine-labeled Tax, which was synthesized by in vitro translation using the TNT-coupled transcription-translation system (Promega). The binding reactions were carried out in 600 ml of GST binding buffer (20 ml Tris-HCl (pH 8.0), 150 mM NaCl, 5% glycerol, 1% Nonidet P-40, 0.5 mM EDTA, and 1 mM dithiothreitol, supplemented with a Complete protease inhibitor mixture tablet (Roche Applied Science)) for 2 h at 4 °C. The beads were washed three times with 1 ml of GST binding buffer. The bound proteins were eluted by adding 20 ml of sample buffer and resolved by SDS-PAGE and then analyzed by autoradiography.

Co-immunoprecipitation Assay—Co-immunoprecipitation assays were performed using HEK-293T cells. The cells were lysed with radiolabeled immunoprecipitation buffer (50 mM Tris-HCl (pH 8.0), 50 mM NaCl, 1 mM EDTA, 0.5% Nonidet P-40, and 1 mM dithiothreitol, supplemented with a Complete protease inhibitor mixture tablet). Cell debris was removed by centrifugation at 10,000 × g for 5 min. The lysates were mixed with protein G beads for 15 min and then incubated with 2 μg of anti-Tax for 2 h at 4 °C and incubated for another 1 h following the addition of 20 ml of protein G bead slurry. The beads were then washed three times with radiolabeled immunoprecipitation buffer, and immunoprecipitates were resolved by SDS-PAGE and analyzed by Western blot.

Luciferase Reporter Assay—Luciferase assays were performed with the dual luciferase reporter assay system (Promega) according to the manufacturer’s instructions. Luciferase activities in cell lysates were normalized relative to the Renilla luciferase activity derived from co-transfected pRL-RSV-luc (31). All reporter assays were performed in triplicate, and standard errors (S.E.) were denoted by the bars in the figures.

RESULTS

TORC3 Activates Transcription at the HTLV-I LTR as Well as Cellular CRE-containing Promoters—Since TORC3 is a co-activator of the transcription factor CREB that enhances CRE-mediated transcription of target genes, we explored the effect of TORC3 on Tax-dependent transcription from the CRE-contain-
consensus sequence, which is
Arrows indicate the TxRE. pCRE-luc contains four copies of the CRE
and flanking GC-rich sequences.

(a)

(b)

FIG. 1.

TORC3 dramatically enhances Tax-mediated transcription
at the HTLV-I LTR as well as pTxRE-luc. A and C, schematic
structures of the reporter plasmids. pLTR-luc consists of the HTLV-I
LTR, designated by U3, R, and U5, followed by the luciferase gene. Arrows indicate the TxRE. pCRE-luc contains four copies of the CRE
consensus sequence, which is underlined. pTxRE-luc has five copies of
the TxRE, which contains an imperfect CRE core element (underlined)
and flanking GC-rich sequences. B, results of a luciferase assay for
pLTR-luc. HEK-293T cells were seeded onto 24-well dishes. Cells were
transfected with 50 ng of pcDNA3-Tax, 50 ng of pcDNA3-HA-TORC3,
and 50 ng of pcDNA3-Tax along with 50 ng of pcDNA3-HA-TORC3,
which have four repeats of the CRE consensus sequence (Fig.
1C). TORC3 alone activates pCRE-luc ∼200-fold above un-
transfected control cells, whereas Tax was unable to activate
transcription from pCRE-luc (Fig. 1D). However, co-expression of
Tax and TORC3 in HEK-293T cells greatly enhanced the
expression of luciferase from pCRE-luc (Fig. 1D), suggesting a
synergistic interaction between these two factors that may
promote high levels of transcription in a cellular context.

Since Tax potentiates the binding of CREB to the TxRE in
the HTLV-I 5′-LTR to initiate viral gene expression, we next
performed a reporter assay using pTxRE-luc. This reporter
plasmid has in its promoter region five copies of the Tax-
responsive element, which contains a core CRE element
flanked by GC-rich sequences (Fig. 1C). In HEK293T cells
transiently expressing TORC3 alone, transactivation of the
HTLV-I LTR reached levels ∼700-fold above background (Fig.
1B). Intriguingly, co-expression of Tax and TORC3 dramati-
cally enhanced pTxRE-luc expression to over 5,000-fold above
background (Fig. 1D). These results indicate that although
TORC3 alone can enhance transcription from the HTLV-I LTR
via the TxRE, Tax is able to synergize with TORC3 to further
increase transcription levels.

**Determination of the Region of TORC3 Important for En-
hancement of Tax-mediated Transcription.** To identify the
region required for Tax-mediated transcription, we generated
several deletion mutants of TORC3 (Fig. 2A) and examined the
effect of these mutant proteins on Tax-mediated transcription.
When HEK-293T cells were co-transfected with TORC3 (1–298
aa) and Tax, the level of the reporter activity remained at
two-thirds of that of cells expressing Tax and full-length
TORC3 (Fig. 2B). However, when TORC3 (298–620 aa) or
TORC3 (95–620 aa), which lack the N-terminal region of
TORC3, were co-transfected with Tax, the reporter activity was
at levels comparable with those observed in cells expressing
Tax alone (Fig. 2B). These results suggest that the N-terminal
region of TORC3 is involved in Tax-mediated transactivation
via the TxRE element. In fact, TORC3 (1–103 aa), which con-
tains only the N-terminal region, showed significant activity.

**TORC3 Interacts with Tax to Promote the Transactivation
via the CRE Sequence.** The N-terminal region of TORC3 con-
tains a coiled-coil domain (∼70 aa), a motif that has often been
implicated in protein-protein interactions. Since TORC3 is a
CREB-interacting protein, we hypothesized that TORC3 en-
hancement of transcription is mediated through its association
with Tax. To test this possibility, we performed a GST pul-
down assay using bacterially generated GST-TORC3 fusion
proteins and in vitro-translated Tax protein. As shown in Fig.
2C, GST-TORC3, but not GST alone, was able to associate with
[35S]methionine-labeled Tax (Fig. 2C, lanes 2 and 3). We found
that Tax was able to bind to the region of TORC3 containing
evaluated with the dual luciferase reporter assay system. D, the effects
of TORC3 on transactivation via the CRE sequence. HEK-293T cells
were transfected with 10 ng of pTxRE-luc (open bars) or 10 ng of
pCRE-luc (solid bars) together with effector plasmids expressing noth-
ing, TORC3, Tax, or both Tax and TORC3. At 24 h after the transfec-
tion, luciferase activities in cell lysates were measured. Average values
of three independent experiments are shown, and standard error (S.E.)
is denoted by the error bars.
Fig. 2. TORC3 interacts with Tax through its N-terminal region, which contains a coiled-coil domain. A, a schematic representation of TORC3 and its deletion mutants. The N-terminal black bar shows the coiled-coil domain. B, the effects of TORC3 and deletion mutants on Tax-mediated LTR transactivation. HEK-293T cells were co-transfected with 10 ng of pLTR-luc and an empty plasmid (−) or plasmids expressing TORC3 (full-length), TORC3 (1–298 aa), TORC3 (298–620 aa), TORC3 (1–103 aa), or TORC3 (95–620 aa) together with (open bar) or without (solid bar) a plasmid expressing Tax. At 24 h after transfection, luciferase activities in cell lysates were measured. Relative luciferase activity in cell lysates with and without Tax expression was normalized to that of the cell lysate without Tax and TORC3 expression. Expression levels of HA-TORC3 and its mutants were examined by Western blotting with an anti-HA antibody. Average values of three independent experiments are shown, and standard error (S.E.) is denoted by the error bars. C, in vitro-translated full-length [35S]methionine-labeled Tax was incubated with GST and GST-TORC3 derivatives, which were immobilized on glutathione-Sepharose beads. The pull-down complexes were analyzed by SDS-PAGE followed by autoradiography. Input corresponds to 10% of the total amount of the reaction mixture. D, HEK-293T cells were transfected with 2 μg of pcDNA3-Myc-TORC3 together with (lane 2) or without (lane 1) 2 μg of pcDNA3-Tax. At 36 h after transfection, cell lysates were immunoprecipitated (IP) by an anti-Tax antibody, and co-immunoprecipitated proteins were resolved by SDS-PAGE and analyzed by Western blot with an anti-Myc antibody.

the coiled-coil domain (Fig. 2C, lanes 4 and 6) but was not able to interact with GST-TORC3 (95–620 aa) or GST-TORC3 (298–620 aa), both of which lacked the coiled-coil domain (Fig. 2C, lanes 5 and 7).

Next, to analyze the interaction of TORC3 with Tax in cells, co-immunoprecipitation experiments were performed. HEK-293T cells were co-transfected with expression plasmids encoding Tax and Myc-tagged TORC3. The immunocomplex precipitated with the anti-Tax antibody was resolved by SDS-PAGE and analyzed by Western blot using the anti-Myc antibody. A 78-kDa band corresponding to the expected size of TORC3 was detected (Fig. 2D, lane 2), suggesting that TORC3 does in fact interact with Tax in cells. This result, together with the data shown above, suggests that the interaction of TORC3 with Tax is required to enhance transcription synergistically via CRE promoter sequences.

**TORC3 Functions as a Co-activator for Tax-mediated Transcriptional Activation**—To examine whether TORC3 has effects on Tax-dependent transcription activity, we constructed an expression plasmid encoding a GAL4 DNA-binding domain-Tax fusion protein, GAL4-Tax, as well as a luciferase reporter plasmid, pGL3-luc, which contains five GAL4-binding sites and an SV40 minimal promoter. These plasmids were introduced into HEK-293T cells together with plasmids expressing full-length TORC3 or TORC3 deletion mutants (Fig. 3). Expression of GAL4-Tax alone is able to activate pGL3-luc. However, co-expression of GAL4-Tax and full-length TORC3 induced a 3-fold increase in pGL3-luc activation as compared with GAL4-Tax alone. Co-expression of GAL4-Tax and TORC3 (1–298 aa) also resulted in enhanced luciferase activity, whereas we observed no synergy in cells co-expressing GAL4-Tax and TORC3 (298–620 aa). These results indicate that the interaction between Tax and TORC3 allows TORC3 to function as a co-activator for Tax.

**Gene Silencing of TORC3 by siRNA Reduces Tax-mediated Transcriptional Activation**—To examine the function of
TORC3 under physiological conditions, we employed siRNA technology, using a synthetic oligonucleotide that forms a duplex RNA encoding nucleotides 1638–1646 of TORC3. Treatment with TORC3 siRNA efficiently reduced the level of TORC3 mRNA in HEK-293T cells to 10% of the control level, whereas the level of GAPDH RNA remained unchanged (Fig. 4A). TORC3 siRNA treatment also resulted in an approximately two-thirds decrease in Tax-mediated transactivation, as measured by pTxRE-luc and pLTR-luc expression (Fig. 4B and C). The values of luciferase activity after 48 h of transfection were almost same as those observed at 24 h (data not shown). Taken together, these data suggest that TORC3 plays an important role in Tax-mediated transactivation of the HTLV-I LTR.

DISCUSSION

The identification of TORCs as CREB modulators (28) prompted us to test the effects of TORC3, which is expressed primarily in B and T lymphocytes (29), on CRE-dependent HTLV-I gene expression induced by Tax. Since the TxRE of the HTLV-I LTR contains a CRE sequence, we examined the effect of TORC3 on transcription of a reporter plasmid containing TxRE in its promoter. We found that although TORC3 was able to enhance transcriptional activation (Fig. 1D), co-expression of Tax with TORC3 resulted in a dramatic increase in expression level. Furthermore, HEK-293T cells co-transfected with Tax and a truncated version of TORC3 (1–298 aa) had a similar level of pTxRE-luc transcription as cells co-expressing full-length TORC3 and Tax. However, co-transfection of Tax with either TORC3 (298–620 aa) or TORC3 (95–620 aa), truncated proteins that lack the N-terminal region, yielded luciferase expression levels similar to those of cells expressing Tax alone (Fig. 2B). We further demonstrated that Tax interacts with the N-terminal region of TORC3, which contains a coiled-coil domain (Fig. 2, C and D). Together, these results suggest that TORC3 enhances CREB activity via CRE sequences through interaction with Tax.

Based on these results, we suggest that TORC3 is included in a complex composed of Tax, CREB, and the viral promoter sequence TxRE. Furthermore, the fact that TORC3 enhanced transcription of a cellular gene containing a CRE element in the promoter and that Tax was able to further enhance this

FIG. 3. Tax-dependent transcription is enhanced by TORC3. HEK-293T cells were cotransfected with 25 ng of GAL4-luciferase reporter plasmid, and 10 ng of the plasmids expressing the GAL4 DNA-binding domain (indicated by GAL4) or the GAL4 DNA-binding domain-Tax fusion protein (indicated by GAL4-Tax) was co-transfected with 100 ng of the plasmid expressing the full-length TORC3, TORC3 (1–298 aa), TORC3 (298–620 aa), or an empty plasmid. At 24 h after transfection, luciferase activity was measured. Relative luciferase activities normalized to those of the cell lysate from cells transfected with pGAL4-luc, an empty plasmid, and pcDNA3 are indicated by a minus sign.

FIG. 4. Tax-mediated transcription depends on endogenous TORC3. A, suppression of endogenous TORC3 by siRNA. Duplexes of 21-nucleotide TORC3 siRNA and control siRNA (random 21-nucleotide sequences) were transfected into HEK-293T cells in 6-cm dishes. TORC3 mRNA levels were examined by RT-PCR at 24 h after transfection. B and C, for the reporter assay, cells were transfected with TORC3 siRNA (solid bar) or control siRNA (open bar). At 24 h after transfection, 50 ng of reporter plasmid (pTxRE-luc or pLTR-luc) and 100 ng of Tax plasmid with (+) or without (−) were transfected into HEK-293T cells. As a control experiment, a reporter assay using pTK-luc was also conducted in the same conditions used for pTxRE-luc. At 24 h after transfection, luciferase activity was measured. Relative luciferase activities normalized to those of control cell lysates without ectopic Tax and TORC3 expression are shown. Average values of three independent experiments are shown, and standard error (S.E.) is denoted by the error bars.
activation (Fig. 1D) also suggest that a Tax/CREB/TORC3 complex associates with cellular CRE sequences to induce transcription. Interestingly, previous work showed that Tax interacts weakly with CREB-2 bound to a cellular CRE motif in the somatostatin promoter (32). It is thus possible that TORC3 has the general function of enhancing transcription of genes with CRE sequences in their promoters and that the level of enhancement depends at least in part on the level of TORC3 in cells. Since TORC2 and TORC3 are predominantly expressed in lymphocytes (29), it is likely that HTLV-I-infected T lymphocytes have the potential to activate genes with CRE-containing promoters.

Furthermore, RNA interference experiments indicated that in cells treated with TORC3 siRNA, Tax-dependent transcription was reduced to less than a third of that in cells treated with control siRNA. This result suggests that Tax requires TORC3 for transcriptional activation of genes carrying TxRE in the promoter, including the HTLV-I LTR, and that TORC3 is an essential component of the Tax/CREB/TxRE ternary complex.

The mechanism by which TORC3 and Tax act synergistically to activate CREB-mediated expression at the HTLV-I LTR remains unclear. However, it has been reported that CBP/p300 and p300/CREB-binding protein-associated factor can be recruited to the HTLV-I TxRE through direct interaction with Tax (7, 21, 22, 33). Thus, it is possible that in vivo, a tetrameric complex of Tax/CREB/TORC3/TxRE may recruit additional co-activator complexes to regulate transcription.

Interestingly, although we identified TORC3 (1–103 aa) as the minimal Tax-binding region, we could detect only a slight enhancement in the level of Tax-mediated transcription as compared with that of TORC3 (1–298 aa) and full-length TORC3 (Fig. 2B). This observation raises questions about how TORC3 induces such a dramatic enhancement in Tax-mediated transcription. In our reporter assay using GAL4-Tax fusion protein, TORC3 (1–298 aa) caused slightly elevated activation compared with that of TORC3 (1–298 aa) and full-length TORC3 (1–298 aa) and that the level of enhancement depends at least in part on the level of TORC3 in cells. Since TORC2 and TORC3 are predominantly expressed in lymphocytes (29), it is likely that HTLV-I-infected T lymphocytes have the potential to activate genes with CRE-containing promoters.

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