Stimulation of IKK-γ oligomerization by the human T-cell leukemia virus oncoprotein Tax

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Received 23 September 2002; revised 5 October 2002; accepted 8 October 2002

First published online 23 October 2002

Edited by Gianni Cesareni

Abstract Human T-cell leukemia virus type 1 oncoprotein Tax activates NF-κB through direct binding to IKK-γ, the regulatory component of the IκB kinase complex. Mechanisms by which IKK-γ adapts the Tax signal to the IκB kinase are poorly understood. Here we demonstrate that IKK-γ forms homodimer and homotrimer both in vitro and in yeast or mammalian cells through a C-terminal domain comprising amino acids 251–419. In contrast, Tax protein targets a central region of IKK-γ, which consists of amino acids 201–250. Interestingly, Tax stimulates the oligomerization of IKK-γ, likely through direct binding. Taken together, our findings suggest a new model of Tax activation of NF-κB, in which Tax interacts with IKK-γ to stimulate its oligomerization.

Key words: Human T-cell leukemia virus type 1 (HTLV-1); Tax oncoprotein; NF-κB; IKK-γ/NEMO; IκB-α phosphorylation; IκB kinase (IKK)

1. Introduction

Human T-cell leukemia virus type 1 (HTLV-1) is the causative agent of adult T-cell leukemia [1,2]. HTLV-1 encodes a 40-kDa oncoprotein Tax, whose expression is required and sufficient for immortalization of T-lymphocytes [3,4], transformation of murine fibroblasts [5], and formation of tumors in transgenic mice [6,7]. Tax potently activates transcription driven by the viral long terminal repeat and it regulates various cellular genes responsive to NF-κB, CREB, and SRF [2,8]. In addition, Tax modulates cell cycle progression and subverts several checkpoints that monitor DNA damage and spindle assembly [2,9–13].

NF-κB represents a major cellular signaling pathway targeted by Tax [14–16]. Through NF-κB, Tax dysregulates the expression of various cytokines and proto-oncogenes. One mechanism by which Tax activates NF-κB involves direct interaction with IKK-γ, also known as NEMO [17], the regulatory component of the IκB kinase (IKK) complex [18–21]. It is thought that IKK-γ adapts a Tax-specific activation signal to IKK-α and IKK-β, which catalyze the phosphorylation of IκB-α leading to its ubiquitination and degradation followed by nuclear translocation and activation of NF-κB [22]. However, it is poorly understood exactly how Tax interaction with IKK-γ activates IKK and NF-κB.

IKK-γ is capable of recruiting IKK-α, IKK-β and the IκB proteins to the IKK complex [23,24]. In addition, emerging evidence has suggested induced oligomerization of IKK-γ as a mechanism for adapting the activation signal from upstream stimulators to IKKs [25–28]. In light of this, here we investigate the influence of Tax on IKK-γ oligomerization in the model organism *Saccharomyces cerevisiae* and in cultured mammalian cells. We show that IKK-γ dimerizes and trimerizes both in vitro and in yeast or mammalian cells. In addition, Tax stimulates IKK-γ oligomerization likely through direct binding. We propose a new model of Tax activation of NF-κB in which Tax-induced oligomerization of IKK-γ represents a critical step.

2. Materials and methods

2.1. Expression and Purification of Histidine-tagged IKK-γ

A DNA fragment containing human IKK-γ cDNA [29,30] was ligated into expression vector pTRCHis (Invitrogen). Histidine-tagged IKK-γ was expressed in *Escherichia coli* BL21 (DE3) and purified using procedures recommended by Invitrogen. His-IKK-γ was eluted from Probond resin with a buffer containing 200 mM imidazole. Proteins were analyzed on 10% SDS–PAGE. Western blotting was performed with a mouse anti-IKK-γ antibody (Transduction Laboratories).

2.2. Chemical cross-linking of purified IKK-γ

Purified recombinant IKK-γ (approximately 2 μg in 10 μl) was incubated with 1 μl of freshly prepared 100 μM bis(sulfosuccinimidyl) suberate (Pierce Biotechnology) at ambient temperature for 15 min. The reaction was stopped with the addition of 1 M Tris (pH 7.5).

2.3. Yeast two-hybrid assay

Yeast two-hybrid assays based on Gal4 DNA-binding domain and Gal4 activation domain (BD and AD) fusions were carried out in strains SFY526 and Y187 as previously described [31,32]. cDNAs coding for IKK-γ mutants were generated by PCR and verified by DNA sequencing. Plasmids for the expression of AD-IKK-γ, BD-Tax, AD-Tax and AD-Int6 in yeast have been described [31–34]. For the expression of BD fusion proteins, cDNAs encoding the IKK-γ mutants were subcloned into plasmid pAS2-1 (Clontech). Details of plasmids and primers will be available upon request.

2.4. Luciferase assay

HeLa cells were propagated in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. JPX9 cells were cultured in RPMI 1640 medium containing 10% fetal calf serum. The expression

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Abbreviations: HTLV-1, human T-cell leukemia virus type 1; IKK, IκB kinase; BD, Gal4 DNA-binding domain; AD, Gal4 activation domain; VP, activation domain of VP16 protein from herpes simplex virus type 1; UAS, upstream activating sequence; GST, glutathione S-transferase; MBP, maltose-binding protein; HA, hemagglutinin

0014-5793/02/$22.00
PII: S0014-5793(02)03590-1

of Tax in JPX9 cells was induced by adding 25 μM $CdSO_4$ to the culture for 3 h. For luciferase assay, cells were grown in six-well culture plates and transfected with Lipofectamine 2000 (Invitrogen). Luciferase activity was measured as described [33] using the Dual-Luciferase® reagents (Promega). Plasmids for mammalian two-hybrid analysis based on Gal4 BD and VP16 activation domain (VP) fusions were from Clontech. Reporter plasmid pGal-LUC as well as expression plasmids for Tax, Tax mutant S258A and hemagglutinin (HA)-tagged IKK-γ have been described [19,33]. Reporter plasmid pκB-LUC was from Stratagene. Transfection efficiencies were normalized with a pRL-TK control reporter (Promega) expressing *Renilla* luciferase.

2.5. Protein interactive assay

Glutathione S-transferase (GST) pull-down assay was performed as described [11,19]. Plasmid pGST-M3 contains the cDNA for M3 mutant of IKK-γ inserted into pGEX4T-1 (Amersham Pharmacia). Plasmid pMBP-Tax has been described [19]. Expression and purification of GST, GST-M3 and maltose-binding protein (MBP)-Tax from *E. coli* were carried out as per protocols recommended by Amersham Pharmacia and New England BioLabs [11,19]. Mouse anti-Tax monoclonal antibody (clone 168A51-42) has been described [11].

BD-IKK-γ and HA-IKK-γ was co-precipitated from extracts of JPX9 cells using a mouse anti-BD antibody (clone RK5C1 from Santa-Cruz). Rabbit polyclonal anti-HA antibody was from Santa-Cruz. Experimental procedures for co-immunoprecipitation have been detailed elsewhere [11].

3. Results

3.1. Dimerization and trimerization of purified IKK-γ

Enforced oligomerization of IKK-γ by fusing it to a FKBP12/Fpk domain that oligomerizes in response to a synthetic organic ligand has been shown to activate IKK and NF-κB [25–27]. In line with this, co-immunoprecipitation of T7-tagged and FLAG-tagged IKK-γ from 293 cells indicates the self-association of IKK-γ [35]. To obtain direct evidence for the oligomerization of IKK-γ, we attempted in vitro chemical cross-linking of purified IKK-γ protein. IKK-γ proteins were treated with bis(sulfosuccinimidyl) suberate, a water-soluble homobifunctional cross-linker, and then analyzed by SDS–PAGE and Western blotting (Fig. 1). Notably, reaction of IKK-γ with the cross-linker yielded two additional species with molecular weights corresponding to IKK-γ dimer and trimer, respectively (Fig. 1, compare lane 2 to lane 1). A

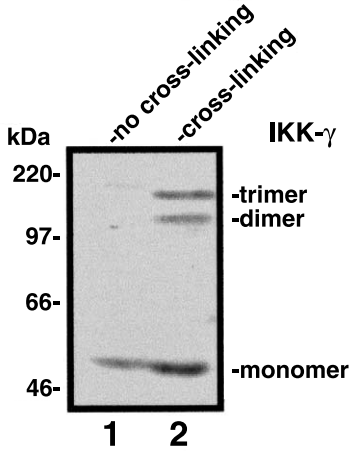

Fig. 1. Dimerization and trimerization of IKK-γ. His-tagged IKK-γ was purified from *E. coli*, chemically cross-linked in vitro, and analyzed by SDS–PAGE and Western blotting with an anti-IKK-γ antibody.

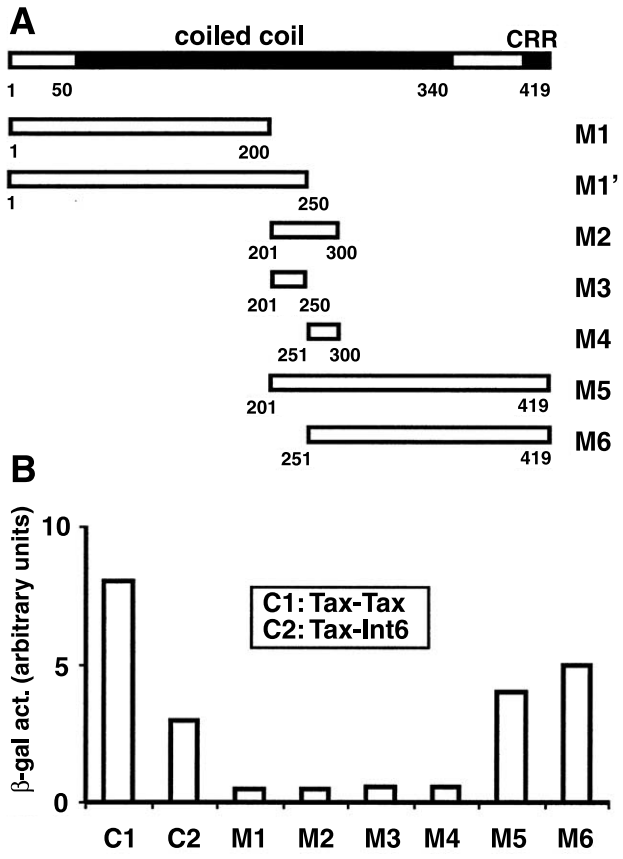

Fig. 2. Oligomerization of IKK-γ through a C-terminal domain. A: Diagram of IKK-γ truncated mutants. The coiled coil region is predicted as described elsewhere [32]. CRR: cysteine-rich region. B: Mapping of the oligomerization domain. Intersubunit interaction was assessed by yeast two-hybrid assay. Two previously reported [31,33] interactive pairs (C1: BD-Tax+AD-Tax; C2: BD-Tax+AD-Int6) are shown as positive controls. SFY526 yeast cells co-transformed with plasmids expressing AD-IKK-γ and BD fusions of the indicated IKK-γ mutants (M1–M6) were measured for β-galactosidase activity (β-gal act.). Similar results were obtained in three independent experiments.

protein band consistent with the size of IKK-γ monomer was observed in both untreated (Fig. 1, lane 1) and treated (Fig. 1, lane 2) samples. Our interpretation of these data is that a fraction of IKK-γ forms dimer and trimer, which coexist with monomer. The three forms are probably interchangeable and in equilibrium.

3.2. IKK-γ oligomerizes through a C-terminal domain

A previous study based on co-immunoprecipitation has mapped the oligomerization domain of human IKK-γ to residues 201–300 [35]. A more recent report based on in vitro assay of recombinant protein suggests that residues 242–388 of mouse IKK-γ harbors the oligomerization domain [28]. With this in mind, we took a different approach to defining the oligomerization domain of IKK-γ.

A complete functional reconstitution of human IKK complex has been demonstrated in budding yeast and this reconstituted system has been successfully used to study the role of IKK-γ on the assembly and activity of IKK complex [36]. IKK activity in yeast affords a rapid and powerful means of dissecting molecular interactions within eukaryotic cells. To

define the oligomerization of IKK-γ in detail, we used reporter yeast strain SFY526 that has been engineered to switch on β-galactosidase expression in response to Gal4 binding to upstream activating sequence (UAS) and subsequent activation of transcription. The yeasts were co-transformed with AD-IKK-γ plasmid and a plasmid expressing BD fusions of various truncated mutants (M1–M6) of IKK-γ, as illustrated in Fig. 2A. The rationale is that when AD-IKK-γ physically interacts with BD-IKK-γ mutant, a strong Gal4 activation domain will be brought proximal to the promoter, thereby potently activating the expression of β-galactosidase reporter.

We observed that the β-galactosidase activities induced by BD-IKK-γ M5 and AD-IKK-γ or by BD-IKK-γ M6 and AD-IKK-γ were comparable to those from two previously reported [31,33] interactive pairs BD-Tax plus AD-Tax and BD-Tax plus AD-Int6 (Fig. 2B). Thus, wild-type IKK-γ containing residues 1–419 interacts with IKK-γ M6 comprising residues 251–419, whereas residues 1–250 are dispensable for self-association of IKK-γ. That is to say, oligomerization of IKK-γ is likely mediated through residues 251–419. Our results are generally consistent with the recent finding that IKK-γ oligomerization domain maps to 242–388 [28].

3.3. Tax targets residues 201–250 of IKK-γ

We and others have shown that HTLV-I Tax activates IKK and NF-κB through direct interaction with IKK-γ [18–21]. It is also understood that Tax protein is functional in yeast [31,32,37]. Thus we sought to define the Tax-binding domain in IKK-γ by using the yeast interactive assay. For this assay, we co-expressed BD-Tax and AD fusion proteins of various IKK-γ mutants in the reporter yeast (Fig. 3A). We noted that the M3 mutant of IKK-γ comprising residues 201–250 and the other two mutants M1′ and M5 containing the same region were capable of binding to Tax. In this setting, these IKK-γ mutants interacts with Tax with potency comparable to the full-length IKK-γ. Hence, amino acid residues 201–250 of IKK-γ sufficiently mediate the interaction between IKK-γ and Tax. We do not fully understand why the M2 mutant is inactive in this assay. One possibility could be that the AD-M2 fusion protein is not properly expressed or structured in the yeast cell.

To verify that residues 201–250 are responsible for binding to Tax in mammalian cells, we asked whether and how the M3 mutant might influence Tax-induced activation of NF-κB in HeLa cells (Fig. 3B). If M3 is not the Tax-binding domain, its expression should have minimal effect on Tax activation of NF-κB. On the contrary, if it does interact with Tax, M3, which lacks other functional domains to activate IKK and NF-κB, would probably squelch active Tax leading to a shut-down of NF-κB activation. Indeed, we observed that the co-expression of M3 with Tax led to significant and dose-dependent inhibition of NF-κB activity induced by Tax (Fig. 3B, compare columns 4 and 5 to column 2). Generally consistent with our previous data [30], the overexpression of IKK-γ enhanced the Tax-dependent activation of NF-κB (Fig. 3B, compare column 3 to column 2).

The above results from both yeast and mammalian cells pointed to an interaction between Tax and residues 201–250

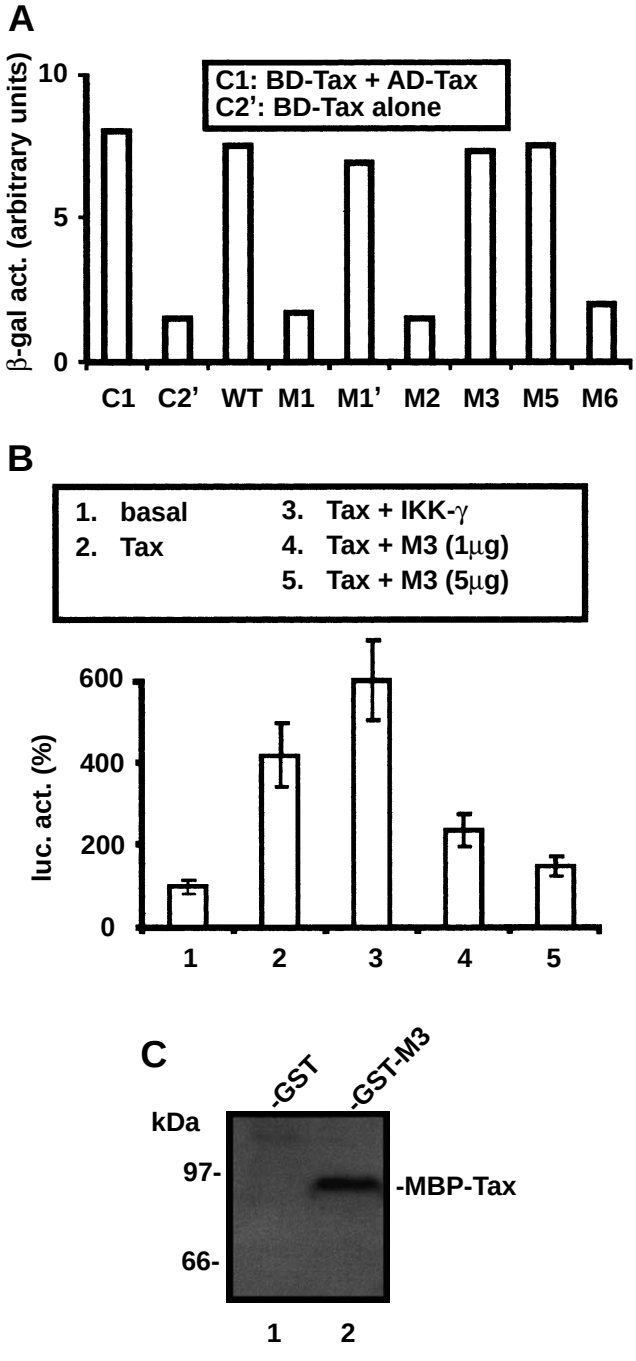

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Fig. 3. Tax interacts with amino acids 201–250 of IKK-γ. A: Yeast two-hybrid assay. Protein–protein interactions were assessed as in Fig. 2. Two control groups (C1: BD-Tax+AD-Tax; C2′: BD-Tax alone) were included. SFY526 yeast cells were co-transformed with plasmids expressing BD-Tax plus AD-IKK-γ (WT: wild-type), or plus the AD fusions of the indicated IKK-γ mutants as defined in Fig. 2A. Similar results were obtained in three independent experiments. B: Repression of Tax activation of NF-κB by the M3 mutant. Reporter plasmid pκB-LUC and plasmids expressing the indicated combinations of proteins were co-transfected into HeLa cells and the luciferase activity (luc. act.) was measured. The summed total amounts of plasmids were normalized in all transfections. Cell transfections were normalized in all experiments by use of a pRL-TK reporter plasmid, which serves as an internal control. Results are representative of three independent experiments. C: GST pull-down assay. GST (lane 1) and GST-M3 (lane 2) proteins were bound to Sepharose beads. Beads were incubated with MBP-Tax, and bound proteins were then eluted with 0.5M KCl. Eluates were analyzed by Western blotting with mouse anti-Tax.

of IKK-γ. To obtain evidence for direct physical contact between these two entities, we performed in vitro pull-down assay using recombinant GST-M3 and MBP-Tax proteins purified from *E. coli*. As shown in Fig. 3C, MBP-Tax bound to GST-M3 (lane 2) but not to GST alone (lane 1). Thus, all three lines of data consistently support a direct interaction between Tax and residues 201–250 of IKK-γ.

3.4. Tax stimulates IKK-γ oligomerization in mammalian cells

Tax functions optimally as a homodimer [31,38]. Tax has also been demonstrated to facilitate dimerization of various bZIP transcription factors leading to enhancement of DNA binding and thereby stimulation of transcription [39–41]. Previously we have proposed a pleiotropic mechanism in which Tax enhances the oligomerization of its various partners [32]. The proposed model and the related findings prompted us to test whether Tax might influence the oligomerization of IKK-γ.

By analogy with yeast two-hybrid analysis, mammalian two-hybrid assay can also be used to study protein–protein interaction in vivo. To explore IKK-γ oligomerization and how this might be influenced by Tax, we designed a modified two-hybrid assay in cultured mammalian cells. We co-transfected into HeLa cells a pGal-LUC reporter plasmid and plasmids expressing Gal4-binding domain-IKK-γ (BD-IKK-γ) and VP16 activation domain-IKK-γ (VP-IKK-γ) fusion proteins. A well-characterized pair of interactive partners (BD-p53+VP-T/SV40) was assayed as a positive control (Fig. 4A, column 1). Notably, the co-expression of VP-IKK-γ with BD-IKK-γ conferred a five-fold increase in the read-out of luciferase activity (Fig. 4A, compare column 4 to column 2), suggesting that the strong activation domain of VP16 was brought proximal to the promoter through intersubunit interaction of the IKK-γ oligomer. As a control, either the expression of BD-IKK-γ alone or the co-expression of BD-IKK-γ and Tax, which is a poor activator in yeast [31], was unable to activate transcription significantly due to the lack of strong activation domain (Fig. 4A, columns 2 and 3). Consistent with data from yeast two-hybrid analysis (Fig. 2), the M6 mutant of IKK-γ was capable of forming oligomer (Fig. 4A, column 7), lending further support to the notion that IKK-γ oligomerizes through a C-terminal domain comprising residues 251–419.

Interestingly, the expression of Tax further stimulated the luciferase activity induced by BD-IKK-γ and VP-IKK-γ by more than two-fold (Fig. 4A, compare column 5 to column 4), suggesting that Tax might enhance IKK-γ oligomerization. This stimulatory effect is not observed with the S258A mutant of Tax, which has been shown previously [19] to be defective for binding to IKK-γ (Fig. 4A, compare column 6 to column 4). In addition, Tax was unable to enhance the oligomerization of the M6 mutant of IKK-γ (Fig. 4A, compare column 8 to column 7), which does not contain a Tax-binding domain. Thus, Tax stimulation of IKK-γ oligomerization is dependent on the physical interaction between Tax and IKK-γ.

To confirm the above findings, we took an independent approach by expressing differentially tagged IKK-γ in JPX9 cells. JPX9 cells were derived from Jurkat T-lymphocytes and the expression of Tax protein in JPX9 can be induced by the addition of Cd^{2+} to the culture [42]. BD-IKK-γ and HA-IKK-γ were co-expressed in un-induced and induced JPX9 cells. The oligomerization of IKK-γ was then assessed by precipitation with a mouse anti-BD antibody followed by Western blotting with a rabbit anti-HA antibody (Fig. 4B). We noted that the induced expression of Tax conferred a 2.3-fold increase in the amount of HA-IKK-γ recovered from the anti-BD immunoprecipitate (Fig. 4B, compare lane

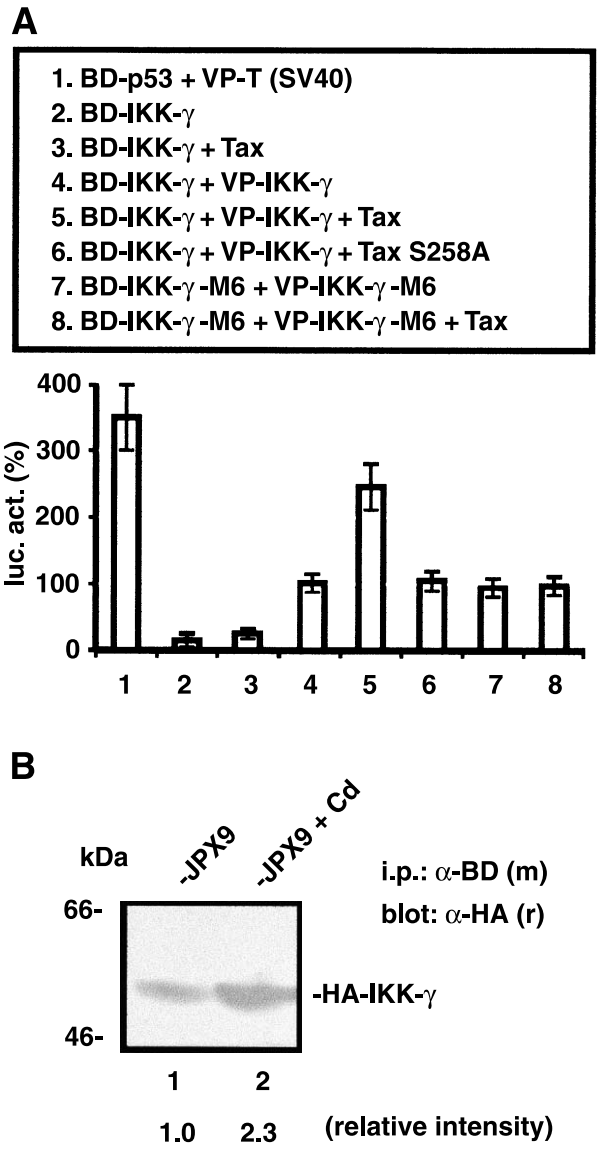

Fig. 4. Tax stimulates IKK-γ oligomerization in cultured mammalian cells. A: Mammalian two-hybrid assay. Reporter plasmid pGal-LUC and plasmids expressing the indicated combinations of proteins were co-transfected into HeLa cells and the luciferase activity (luc. act.) was measured. The summed total amounts of plasmids were normalized in all transfections. Cell transfections were normalized in all experiments by use of a pRL-TK reporter plasmid, which serves as an internal control. Results are representative of three independent experiments. B: Increased oligomerization of IKK-γ upon expression of Tax. Plasmids expressing BD-IKK-γ and HA-IKK-γ were transiently transfected into JPX9 cells by electroporation. Induction of Tax expression was achieved by adding 25 μM $CdSO_4$ to the culture for 3 h. Extracts of un-induced (lane 1) and Cd^{2+}-induced (lane 2) JPX9 cells were immunoprecipitated with mouse anti-BD antibody. The precipitates were probed by Western blotting with rabbit anti-HA. Relative intensities of the HA-IKK-γ band, as shown at the bottom, are determined by a densitometer. In separate control experiments, the addition of Cd^{2+} to Tax-non-expressing Jurkat cells did not affect IKK-γ oligomerization (data not shown).

2 to lane 1), suggesting that significantly more protein complexes comprising BD-IKK-γ and HA-IKK-γ had been formed in the cells. These results agree generally with the concept that Tax enhances IKK-γ oligomerization.

4. Discussion

We demonstrate that IKK-γ dimerizes and trimerizes in vitro (Fig. 1). The oligomerization of IKK-γ is mediated through a C-terminal domain comprising residues 251–419, as shown in yeasts (Fig. 2) and in cultured mammalian cells (Fig. 4). The HTLV-1 Tax oncoprotein interacts with a central domain of IKK-γ comprising residues 201–250 (Fig. 3). This interaction leads to enhancement of IKK-γ oligomerization (Fig. 4), which is required for the assembly and activation of IKK complex. Our findings suggest a new model for Tax activation of NF-κB in which Tax contacts IKK-γ and facilitates its oligomerization.

We noted that both the Tax-binding and oligomerization domains in IKK-γ overlap with the extended coiled coil region (residues 51–353). Coiled coils represent an evolutionarily conserved motif for protein–protein interaction and a particular coiled coil structure shared by several Tax-binding proteins including IKK-γ is likely recognized by Tax [32]. Interestingly, IKK-γ uses a separate coiled coil subdomain to mediate homo-oligomerization. Thus, protein–protein interactions mediated through coiled coil motifs are highly specific. We also noted that the C-terminal zinc finger domain of IKK-γ has recently been shown to be necessary for full IKK activation in response to tumor necrosis factor-α, UV radiation and topoisomerase inhibitors [43, 44]. In this regard, it would be of interest to see whether the zinc finger is also involved in IKK-γ oligomerization.

Mechanistically, induced oligomerization of IKK-γ likely represents a shared critical step where different upstream signals converge in their activation of IKK and NF-κB. In addition to Tax, other viral and cellular signaling pathways including tumor necrosis factor α, RIP, vCLAP from equine herpesvirus-2, and Nod1/RICK may operate through the same mechanism [25–27]. In this regard, it would be of interest to identify additional NF-κB stimuli that can induce IKK-γ oligomerization.

Acknowledgements: D.-Y.J. is a Leukemia and Lymphoma Society Scholar. This work was supported by National Natural Science Foundation of China (NSFC) Grant 30029001 (to D.-Y.J.) and a matching fund (to D.-Y.J.) from the University Research Committee (URC) of the University of Hong Kong.

References

[1] Uchiyama, T. (1997) Annu. Rev. Immunol. 15, 15–37.
[2] Yoshida, M. (2001) Annu. Rev. Immunol. 19, 475–496.
[3] Grassmann, R., Dengler, C., Muller-Fleckenstein, I., Fleckenstein, B., McGuire, K., Dokhelar, M.C., Sodroski, J.G. and Haseltine, W.A. (1989) Proc. Natl. Acad. Sci. USA 86, 3351–3355.
[4] Akagi, T., Ono, H. and Shimotohno, K. (1995) Blood 86, 4243–4249.
[5] Tanaka, A., Takahashi, C., Yamaoka, S., Nosaka, T., Maki, M. and Hatanaka, M. (1990) Proc. Natl. Acad. Sci. USA 87, 1071–1075.
[6] Nerenberg, M., Hinrichs, S.H., Reynolds, R.K., Khoury, G. and Jay, G. (1987) Science 237, 1324–1329.
[7] Grossman, W.J., Kimata, J.T., Wong, F.H., Zutter, M., Ley, T.J. and Ratner, L. (1995) Proc. Natl. Acad. Sci. USA 92, 1057–1061.
[8] Flint, J. and Shenk, T. (1997) Annu. Rev. Genet. 31, 177–212.
[9] Suzuki, T., Kitao, S., Matsushime, H. and Yoshida, M. (1996) EMBO J. 15, 1607–1614.
[10] Neuveut, C., Low, K.G., Maldarelli, F., Schmitt, I., Majone, F., Grassmann, R. and Jeang, K.-T. (1998) Mol. Cell Biol. 18, 3620–3632.
[11] Jin, D.-Y., Spencer, F. and Jeang, K.-T. (1998) Cell 93, 81–91.
[12] Kasai, T., Iwanaga, Y., Iha, H. and Jeang, K.-T. (2002) J. Biol. Chem. 277, 5187–5193.
[13] Liang, M.-H., Geisbert, T., Yao, Y., Hinrichs, S.H. and Giam, C.-Z. (2002) J. Virol. 76, 4022–4033.
[14] Ballard, D.W., Bohnlein, E., Lowenthal, J.W., Wano, Y., Franza, B.R. and Greene, W.C. (1988) Science 241, 1652–1655.
[15] Sun, S.-C. and Ballard, D.W. (1999) Oncogene 18, 6948–6958.
[16] Jeang, K.-T. (2001) Cytokine Growth Factor Rev. 12, 207–217.
[17] Yamaoka, S., Courtois, G., Bessia, C., Whiteside, S.T., Weil, R., Agou, F., Kirk, H.E., Kay, R.J. and Israël, A. (1998) Cell 93, 1231–1240.
[18] Chu, Z.L., Shin, Y.A., Yang, J.M., DiDonato, J.A. and Ballard, D.W. (1999) J. Biol. Chem. 274, 15297–152300.
[19] Jin, D.-Y., Giordano, V., Kibler, K.V., Nakano, H. and Jeang, K.-T. (1999) J. Biol. Chem. 274, 17402–17405.
[20] Harhaj, E.W. and Sun, S.-C. (1999) J. Biol. Chem. 274, 22911–22914.
[21] Xiao, G., Harhaj, E.W. and Sun, S.-C. (2000) J. Biol. Chem. 275, 34060–34067.
[22] Karin, M. and Ben-Neriah, Y. (2000) Annu. Rev. Immunol. 18, 621–663.
[23] Li, X.-H., Fang, X. and Gaynor, R.B. (2001) J. Biol. Chem. 276, 4494–4500.
[24] Yamamoto, Y., Kim, D.-W., Kwak, Y.-T., Prajapati, S., Verma, U. and Gaynor, R.B. (2001) J. Biol. Chem. 276, 36327–36336.
[25] Inohara, N., Koseki, T., Lin, J., del Peso, L., Lucas, P.C., Chen, F.F., Ogura, Y. and Núñez, G. (2000) J. Biol. Chem. 275, 27823–27831.
[26] Poyet, J.-L., Srinivasula, S.M., Lin, J.-H., Fernandes-Alnemri, T., Yamaoka, S., Tsichlis, P.N. and Alnemri, E.S. (2000) J. Biol. Chem. 275, 37966–37977.
[27] Poyet, J.-L., Srinivasula, S.M. and Alnemri, E.S. (2001) J. Biol. Chem. 276, 3183–3187.
[28] Agou, F., Ye, F., Goffinont, S., Courtois, G., Yamaoka, S., Israël, A. and Véron, M. (2002) J. Biol. Chem. 277, 17464–17475.
[29] Jin, D.-Y. and Jeang, K.-T. (1999) J. Biomed. Sci. 6, 115–120.
[30] Jin, D.-Y., Giordano, V., Kibler, K.V., Nakano, H. and Jeang, K.-T. (1999) J. Biol. Chem. 274, 17402–17405.
[31] Jin, D.-Y. and Jeang, K.-T. (1997) Nucleic Acids Res. 25, 379–387.
[32] Chun, A.C.S., Zhou, Y., Wong, C.-M., Kung, H.-F., Jeang, K.-T. and Jin, D.-Y. (2000) AIDS Res. Hum. Retrov. 16, 1689–1694.
[33] Zhou, H.-J., Wong, C.-M., Chen, J.-H., Qiang, B.-Q., Yuan, J.-G. and Jin, D.-Y. (2001) J. Biol. Chem. 276, 28933–28938.
[34] Neuveut, C., Jin, D.-Y., Semmes, O.J., Diella, F., Callahan, R. and Jeang, K.-T. (1997) J. Biomed. Sci. 4, 229–234.
[35] Ye, J., Xie, X., Tarassishin, L. and Horwitz, M. (2000) J. Biol. Chem. 275, 9882–9889.
[36] Miller, B.S. and Zandi, E. (2001) J. Biol. Chem. 276, 36320–36326.
[37] Wu, L.-C.C., Tan, T.-H. and Shahied, S.I. (1992) J. Virol. 66, 7253–7261.
[38] Tie, F., Adya, N., Greene, W.C. and Giam, C.-Z. (1996) J. Virol. 70, 8368–8374.
[39] Wagner, S. and Green, M.R. (1993) Science 262, 395–399.
[40] Perini, G., Wagner, S. and Green, M.R. (1995) Nature 376, 602–605.
[41] Baranger, A.M., Palmer, C.R., Hamm, M.K., Giebler, H.A., Brauweiler, A., Nyborg, J.K. and Schepartz, A. (1995) Nature 376, 606–608.
[42] Nagata, K., Ohtani, K., Nakamura, M. and Sugamura, K. (1989) J. Virol. 63, 3220–3226.
[43] Huang, T.T., Feinberg, S.L., Suryanarayanan, S. and Miyamoto, S. (2002) Mol. Cell Biol. 22, 5813–5825.
[44] Makris, C., Roberts, J.L. and Karin, M. (2002) Mol. Cell Biol. 22, 6573–6581.