MEKK2 Is Required for T-cell Receptor Signals in JNK Activation and Interleukin-2 Gene Expression*

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The c-Jun N-terminal kinases (JNKs) are members of the mitogen-activated protein kinase (MAPK) gene family and are essential for cell proliferation, differentiation, and apoptosis. Previously we found that activation of JNK in T-cells required costimulation of both T-cell receptor and auxiliary receptors such as CD28. In this study, we cloned a full-length human MEK kinase (MEKK) 2 cDNA from Jurkat T-cells and demonstrated that it was a major upstream MAPK kinase for the JNK cascade in T-cells. The human MEKK2 cDNA encoded a polypeptide of 619 amino acids and was the human counterpart of the reported murine MEKK2. It was 94% homologous with human and murine MEKK3 at the catalytic domains and 60% homologous at the N-terminal noncatalytic region. Northern blot analysis showed that MEKK2 was ubiquitously expressed, with the highest level in peripheral blood leukocytes. In T-cells, MEKK2 was found to be a strong activator of JNK but not of extracellular signal-regulated kinase MAPKs and to activate JNK-dependent AP-1 reporter gene expression. MEKK2 also synergized with anti-CD3 antibody to activate JNK in T-cells, and stimulation of T-cells led to induction of MEKK2 tyrosine phosphorylation. Significantly, the JNK activation induced by anti-CD3 and anti-CD28 antibodies, but not by 12-O-tetradecanoylphorbol-13-acetate and Ca++ ionophore A23187, was inhibited by dominant negative MEKK2 mutants. AP-1 and interleukin-2 reporter gene induction in T-cells was also inhibited by dominant negative MEKK2 mutants. Taken together, our results showed that human MEKK2 is a key signaling molecule for T-cell receptor/CD3-mediated JNK MAPK activation and interleukin-2 gene expression.

T-cell costimulation, and growth factors (1–3). Like all other MAPKs, JNK is activated by phosphorylation of the conserved Thr and Tyr residues by its activating MAPK kinases (MAPKs). Two MAPKks, JNK1/MKK4 and JNK2/MKK7, were identified as participating in JNK activation (4–13). Multiple MAPKK kinases (MAPKKKs) have been shown to utilize JNKK1/MKK4 and JNKK2/MKK7 to activate the JNK cascade (14–17). These MAPKKKs have in common conserved kinase domains with substantial homology to the yeast MAPKKK STE11 (18–25). However, other than the conserved catalytic domains, these kinases in general are not similar and are believed to be involved in transducing different upstream signals to downstream targets (14, 25–28).

It has been shown that during T-cell activation, activation of the JNK, but not of the Erk MAPK, cascade is dependent on costimulation of T-cell receptor (TCR) and the costimulatory receptor CD28 (29). Activation of c-Jun/AP-1, the nuclear target of the JNK MAPK cascade, is also dependent on costimulation of TCR and CD28 in naïve CD4+ T-cells (30). More recently, the role of the JNK MAPK cascade in T-cells was studied in gene-knockout mice, which showed that JNK1 and JNK2 play a crucial role in T-cell activation and differentiation (31–33).

The signal transduction pathways that lead to JNK activation in T-cells remain unknown. Studies in fibroblasts indicated that members of the mitogen-activated protein kinase/extracellular signal-regulated kinase (MEK) kinase (MEKK) gene family are the major MAPKKks for the JNKs (1, 15, 18, 19, 28, 34). MEKK1, the first MEKK identified in mammalian cells, was suggested to be involved in TCR-mediated JNK activation and cytokine gene expression (35). Interestingly, a recent study suggested that MEKK2 rather than MEKK1 was involved in transducing TCR signals in T-cells (36). This study showed that murine MEKK2 but not MEKK1 is translocated to the T-cell/antigen-presenting cell interface during antigen stimulation (36). Whether other members of the MEKK gene subfamily are involved in T-cell signal transduction is not clear. So far, no lymphocyte-specific MAPKKks for the JNK cascade have been identified.

In this study, we described the cloning and characterization of human MEKK2, a potent JNK MAPK activator, in T-cells. We found that MEKK2 was a specific JNK upstream-activating kinase in T-cells and was involved in transducing TCR/CD3-mediated T-cell signals for JNK MAPK activation and T-cell-specific gene expression.

EXPERIMENTAL PROCEDURES

Isolation of Full-length Human MEKK2 cDNA Clone—The degenerate oligonucleotides TTAATGGCNGTNAA(a/g)CA and TTNGCNCC(t/a)(t/g)TG, which corresponded to sequences in kinase subdomains II and VII, respectively, of the yeast MAPKKK STE11 (37) were used to amplify related gene fragments by reverse transcription-polymerase chain reaction (PCR) with mRNA from Jurkat T-cells. A mixture of cDNA fragments of about 300 base pairs was

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amplified and subcloned into the TA cloning vector (Invitrogen, San Diego, CA) for sequencing. We identified a cDNA fragment encoding a polypeptide 47% similar to STE11, 49% similar to murine MEKK1, and 99% similar to murine MEKK2 at the predicted kinase subdomains II-VII. This cDNA fragment was used as a probe to further screen a Jurkat cDNA library. We obtained four cDNA clones that contained a complete putative kinase domain, but all lacked the complete 5’ coding sequence. Full-length cDNA clones were isolated by further screening a human lymphocyte cDNA library (Invitrogen) with PCR with primers that matched the 5’ sequence of the partial cDNA clones isolated from the Jurkat cDNA library. Ten individual clones were isolated, and the longest one, clone 2g, was completely sequenced and used in this study.

**Plasmids, Proteins, and Antibodies**—SRaRasL61, SRaRafBxB, SRaMEKK2, SRaHA-JNK1, SRaHA-Erk2, SRaHA-MEKK2, -73Col-Luc, -79Jun-Luc, IL-2-Luc, and pActin-β-Gal were described previously (2, 28, 29). The hemagglutinin (HA)-tagged MEKK2(NT) expression vector SraHA-MEKK2(NT) was constructed by introducing an NcoI site

![Figure 1. Cloning of human MEKK2.](http://www.jbc.org/Downloadedfrom)
into the first Met codon of MEKK2 by a PCR-based method and subcloning the Ncol-XhoI fragment that encodes amino acids 1–359 into the expression vector pSRαHA. MEKK2(KM) is a MEKK2 mutant with a Lys-385 → Met mutation that was generated by PCR-directed mutagenesis. The MEKK2(KM) mutant cDNA was subcloned into SRαHA as described for SRαHA-MEKK2 (28). Expression and purification of the bacterial fusion protein glutathione S-transferase (GST)-c-Jun-(1–79) was previously described (6, 38). Anti-phosphotyrosine antibody PY20 was purchased from Promega (Madison, WI).

**Northern Blot Analysis**—A multitissue poly(A)⁺ blot was purchased from CLONTECH (Palo Alto, CA) and probed with a 1-kilobase NcoI-XhoI cDNA probe from the N-terminal region of human MEKK2 using the manufacturer’s suggested protocol.

**Cell Culture and Transfection Procedures**—Jurkat T-cells were cultured in RPMI medium supplemented with 10% fetal bovine serum, 1 mM glutamate, 100 units/ml penicillin, and 100 mg/ml streptomycin. COS-1 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 5% fetal bovine serum, 100 units/ml penicillin, and 100 mg/ml streptomycin. Jurkat T-cells were transfected by electroporation as described previously (29). COS-1 cells were transfected with LipofectAMINE (Life Technologies, Inc.).

**In Vitro Kinase Assay**—Cell lysates were prepared 40 h after transfection as previously described (29) and incubated with appropriate antibodies for 4 h at 4°C in a rotator. Then, protein A-Sepharose beads were added, and the mixture was incubated for another 45 min. The beads were washed four times with lysis buffer (20 mM Tris, pH 7.5; 0.5% Nonidet P-40; 250 mM NaCl; 3 mM EDTA; 3 mM EGTA; and 100 mM Na3VO4) and twice with kinase reaction buffer (20 mM HEPES, pH 7.6; 1 mM p-nitrophenyl phosphate; 20 mM MgCl2; 2 mM EDTA; 2 mM EGTA; and 100 μM Na3VO4). The immunoprecipitates were subjected to kinase assays in 30 μl of kinase buffer with appropriate substrates in the presence of 0.5 μl of [γ-32P]ATP and 20 μl of cold ATP. After 20 min at 30°C, the reactions were terminated with SDS-polyacrylamide gel electrophoresis loading buffer and boiled for 5 min. The proteins were separated by SDS-polyacrylamide gel electrophoresis, and 32P incorporation was determined with a Bio-Rad phosphorimaging apparatus (Bio-Rad).

**Luciferase Reporter Gene Assay**—Luciferase assay was described previously (13), and luciferase activity was measured with a luminometer TD-20/20 from Promega (Madison, WI).

**Western Blot**—Western blot with anti-HA antibody 12CA5 and anti-MEKK2 antibodies 1128 and 1129 was performed as previously described using an ECL kit (Amersham Pharmacia Biotech) (28).

**RESULTS**

**Cloning of Human MEKK2**—To isolate a potential MAPKKK gene upstream of the JNK cascade from T-cells, we made degenerated oligonucleotides that corresponded to the sequences of the kinase subdomains II and VII of yeast MAPKKK STE11 (37), because MAPKKK genes were highly conserved from yeast to mammals, and used them to amplify related gene fragments by reverse transcription-PCR of mRNA from Jurkat T-cells. This cDNA fragment with substantial homology to the catalytic domain of STE11, MEKK1, and murine MEKK2 was identified and used as a probe to clone a full-length cDNA from a human lymphocyte cDNA library as described under “Exper-

**MEKK2 in TCR/CD3 Signaling**

![Fig. 2. MEKK2 was a specific upstream activator of JNK activation in T-cells.](http://www.jbc.org/)

A MEKK2 specifically activated JNK but not Erk MAPK in T-cells. One μg of HA-JNK1 and HA-Erk2 expression vectors was cotransfected with control empty vector or expression vectors for active Ras mutant RasL61, active Raf mutant RafBxB, and MEKK2 into Jurkat T-cells as indicated. Thirty-six h after transfection, HA-JNK1 and HA-Erk2 were immunoprecipitated with anti-HA antibody, and their activity was determined by *in vitro* kinase assays (KA) using GST-c-Jun (for JNK) and myelin basic protein (for Erk) as substrates. Expression of HA-JNK1 and HA-Erk2 was measured by Western blot (WB). B, effect of MEKK2 on AP-1 activation in Jurkat T-cells and COS-1 cells. AP-1 reporter gene plasmid -73Col-Luc was cotransfected into Jurkat T-cells (5 μg/transfection) and COS-1 cells (0.5 μg/transfection) with the indicated amount of MEKK2 expression vector. pActin-β-Gal was included in each transfection to monitor the transfection efficiency. The relative luciferase activity was measured 36 h later and normalized to the β-galactosidase activity. The results are presented as the average of three independent experiments. The error bars indicate the standard error.
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The amounts of MEKK2 expression vector in each transfection. Mined with a Bio-Rad phosphorimaging apparatus and plotted against as described in the legend to Fig. 2.

Two antibody 9.3 (0.2 μg/ml) expression vector into 10⁷ Jurkat T-cells. Each transfection mixture was cotransfected with increasing amounts of MEKK2 expression vector was transfected into Jurkat T-cells. As described in the legend to Fig. 2A. Relative JNK activity was determined with a Bio-Rad phosphorimaging apparatus and plotted against the amounts of MEKK2 expression vector in each transfection.

Tyrosine phosphorylation of MEKK2 by CD3 stimulation. Five μg of GST-MEKK2 expression vector was transfected into Jurkat T-cells as described in the legend to Fig. 2A. Thirty-six h after transfection, the cells were incubated with control antibody or anti-CD3 antibody OKT3 (10 μg/ml) for 30 min at 4 °C. Free antibody was removed by washing two times with cold phosphate-buffered saline, and the cells were further treated with cross-linker antibody (Ab) goat anti-mouse IgG (10 μg/ml) for 20 min before harvesting. GST-MEKK2 was precipitated with GST beads and subjected to Western blot (WB) analysis with anti-phosphotyrosine (anti-Pty) antibody. MEKK2 expression was determined by Western blotting with anti-MEKK2 antibody 1128.

MEKK2 Activates the JNK Cascade in T-cells—We recently demonstrated that in fibroblasts human MEKK2 is an important activating kinase of JNK and acts through formation of a specific MAPK module with JNKK2 and JNK1 (28). To investigate whether MEKK2 is also involved in transducing T-cell-activating signals and JNK activation, we expressed full-length human MEKK2 with HA-tagged JNK1 in Jurkat T-cells and analyzed it with an anti-phosphotyrosine antibody. MEKK2 was precipitated with GST beads and subjected to Western blot analysis with anti-phosphotyrosine antibody (Fig. 2A).

Previously, we demonstrated that costimulation of Jurkat T-cells with anti-CD3 and anti-CD28 antibodies led to strong synergistic JNK activation (29). Because MEKK2 was able to activate JNK in T-cells (Fig. 2A), we next examined whether MEKK2 could potentiate JNK1 activation by these T-cell stimuli. To do so, we transfected HA-JNK1 expression vector with increasing amounts of MEKK2 expression vector into Jurkat T-cells and measured the JNK activity without stimulation or with anti-CD3 or anti-CD28 antibody stimulation. As shown in Fig. 3, whereas anti-CD28 stimulation had no effect on MEKK2-mediated JNK activation, anti-CD3 stimulation greatly enhanced the MEKK2-mediated JNK activation, especially at low levels of MEKK2 expression. These results suggested that MEKK2 might be involved in transducing TCR/CD3 signals during T-cell antigen stimulation. The same conclusion was reached in a recent study on murine MEKK2 (36).

Tyrosine Phosphorylation of MEKK2 by CD3 Stimulation—The above results suggested that MEKK2 may act downstream of the TCR/CD3 receptor. To further examine this issue, we tested whether MEKK2 could be directly modified following anti-CD3 antibody stimulation in T-cells. Recently, Scharer et al. (36) found that murine MEKK2 was translocated to the T-cell-antigen-presenting cell interface after antigen stimulation, suggesting that MEKK2 may be an early target of TCR signaling. Because stimulation of TCR rapidly activates several protein tyrosine kinases, it is possible that human MEKK2 may be regulated by tyrosine phosphorylation in T-cells. To test this possibility, we transfected GST-tagged MEKK2 into Jurkat T-cells and analyzed it with an anti-phosphotyrosine antibody after stimulation with anti-CD3 antibody. As shown in Fig. 4, we found that there was a rapid induction of MEKK2 tyrosine phosphorylation in response to CD3 stimulation. We also observed a similar pattern of tyrosine phosphorylation of the endogenous MEKK2, and such phosphorylation was not affected by addition of anti-CD28 antibody (data not shown).

This result indicated that MEKK2 was a substrate molecule for TCR/CD3 activated protein tyrosine kinases.
MEKK2 is Required for JNK Activation by T-cell Costimulation and IL-2 Gene Expression—To further determine the role of MEKK2 in T-cell stimulation, we constructed the expression vector SRA-HA-MEK2(NT), which expresses a MEKK2 mutant, MEKK2(NT), which lacks the entire catalytic domain. Expression of MEKK2(NT) in T-cells inhibited MEKK2-induced JNK activation in a dose-dependent manner (data not shown), suggesting that MEKK2(NT) is a dominant negative mutant. To determine the effect of MEKK2(NT) on JNK activation in T-cells, we transfected HA-JNK1 with increasing amounts of MEKK2(NT) and determined the JNK activity 36 h later with or without T-cell stimulation with anti-CD3 and anti-CD28 antibodies. As shown in Fig. 5A, JNK activation by T-cell costimulation with anti-CD3 and anti-CD28 antibodies was strongly inhibited by MEKK2(NT), suggesting that MEKK2 is required for JNK activation by costimulation with anti-CD3 and anti-CD28 antibodies. Interestingly, in a similar experiment, we found that costimulation of JNK in T-cells by TPA and A23187 was not blocked by the mutant MEKK2(NT) (Fig. 5B). Transfection of MEKK2(NT) into Jurkat cells also did not inhibit the JNK activation in response to a combined stimulation with TPA, A23187, and anti-CD28 antibody (data not shown). Similar results were also reported with murine MEKK2 (36), suggesting that MEKK2 may not be involved in JNK activation by TPA plus Ca²⁺ plus CD28 in T-cells.

Because MEKK2 was required for JNK activation in T-cells, we next examined whether this effect was dependent on MEKK2 catalytic activity. We constructed the expression vector SRA-MEK2(KM), which expresses a MEKK2 mutant, MEKK2(KM), with a point mutation of the conserved Lys-385 to Met in the ATP binding motif. Such a mutation in the ATP binding site has been shown to abolish the catalytic activity of many serine/threonine kinases (14), and by itself MEKK2(KM) did not activate JNK in T-cells (data not shown). We transfected this mutant kinase with HA-JNK1 into Jurkat T-cells and determined the JNK1 activity with or without anti-CD3 and anti-CD28 antibody costimulation. As shown in Fig. 5C, the MEKK2 mutant inhibited JNK activation by CD3 and CD28 costimulation in T-cells. One μg of HA-JNK1 expression vector was cotransfected with increasing amounts of MEKK2(KM) expression vector into 10⁷ Jurkat T-cells. The cells were stimulated with TPA (10 ng/ml) and A23187 (1 μg/ml) for 30 min 36 h after transfection, and the JNK1 activity was assayed as described for A. MEKK2(KM) inhibited JNK1 activation by stimulation of T-cells with anti-CD3 and anti-CD28 antibodies. One μg of HA-JNK1 expression vector was cotransfected with increasing amounts of MEKK2(KM) expression vector into 10⁷ Jurkat T-cells. T-cell stimulation and JNK1 activity assay were carried out as described in A. D, MEKK2(KM) blocked -79Jun-Luc and IL2-Luc reporter gene expression in T-cells. Two μg of -79Jun-Luc reporter plasmid or 5 μg of IL2-Luc reporter plasmid was cotransfected with increasing amounts of MEKK2(KM) expression vector.

Twenty-four h after transfection, the cells were stimulated with 10 μg/ml anti-CD3 antibody OKT3 and 0.2 μg/ml anti-CD28 antibody 9.3 for 12 h before harvesting for the luciferase assay. Luciferase activity was normalized to β-galactosidase activity, and the results shown are the average of three independent experiments. The error bars indicate the standard error.

Fig. 5. MEKK2 was required for JNK1 activation by CD3 and CD28 costimulation in T-cells. A, MEKK2(NT) inhibited JNK activation by T-cell costimulation with anti-CD3 and anti-CD28 antibodies. One μg of HA-JNK1 expression vector was cotransfected with increasing amounts of MEKK2(NT) expression vector into 10⁷ Jurkat T-cells. Thirty-six h after transfection, the cells were stimulated with 10 μg/ml anti-CD3 antibody OKT3 and 0.2 μg/ml anti-CD28 antibody 9.3 for 30 min before harvesting for the JNK1 assay as described previously (29). Relative GST-c-Jun phosphorylation was measured by Western blotting. B, MEKK2(NT) did not inhibit JNK activation by TPA and A23187. One μg of HA-JNK1 expression vector was cotransfected with increasing amounts of MEKK2(NT) expression vector into 10⁷ Jurkat T-cells. The cells were stimulated with TPA (10 ng/ml) and A23187 (1 μg/ml) for 30 min 36 h after transfection, and the JNK1 activity was assayed as described for A. C, MEKK2(KM) inhibited JNK1 activation by costimulation of T-cells with anti-CD3 and anti-CD28 antibodies. Two μg of HA-JNK1 expression vector was cotransfected with increasing amounts of MEKK2(KM) expression vector into 10⁷ Jurkat T-cells. T-cell stimulation and JNK1 activity assay were carried out as described in A. D, MEKK2(KM) blocked -79Jun-Luc and IL2-Luc reporter gene expression in T-cells. Two μg of -79Jun-Luc reporter plasmid or 5 μg of IL2-Luc reporter plasmid was cotransfected with increasing amounts of MEKK2(KM) expression vector.
CD28 costimulation. This result showed that the MEKK2(KM) mutant also had a dominant negative effect on JNK activation in T-cells.

It has been shown that the JNK MAPK cascade is crucial for AP-1 and IL-2 gene expression in T-cells (29, 30). Because MEKK2 was involved in regulating JNK activation in T-cells, it was likely that MEKK2 was also required for the expression of these genes in T-cells. To investigate this, we transfected MEKK2(KM) with the AP-1 reporter plasmid -79Jun-Luc and the IL-2 reporter plasmid IL2-Luc into T-cells and determined the effect on the reporter gene expression after T-cell costimulation. As shown in Fig. 5D, expression of MEKK2(KM) inhibited AP-1 and IL-2 reporter gene induction by T-cell costimulation, suggesting that MEKK2 is crucial for T-cell gene expression.

**DISCUSSION**

A growing number of MAPKKKs, including MEKK1, MEKK2, MEKK3, MEKK4, MEKK5, apoptosis signal-regulating kinase 1, transforming growth factor-β-activated protein kinase 1, and tumor progression locus 2, have been shown to be capable of activating JNK (18, 20–24, 27, 34, 40). These kinases have a conserved kinase domain with substantial homology to that of the yeast MAPKKK STE11. However, how these MAPKKKs are involved in directing different cell surface signals to the downstream cytoplasmic and nuclear effectors in different tissues is still unknown.

In this study, we described the cloning and characterization of human MEKK2, a full-length cDNA from Jurkat T-cells. The MEKK2 cDNA described in this study encoded a serine/threonine kinase and is probably the human counterpart of the reported murine MEKK2 (18), because these two molecules are very similar (96% identical). Both human and murine MEKK2 are much more similar to human and murine MEKK3 at the catalytic domain (94% identical) than are other members of the MEKK gene subfamily, indicating that MEKK2 and MEKK3 are closely related. Interestingly, Northern blot analysis showed that MEKK2 was expressed at high levels in spleen and peripheral blood leukocytes but at low levels in thymus, suggesting that MEKK2 may have an important function in lymphocyte activation but not in normal T-cell development.

Expression of dominant negative MEKK2 mutants was found to block JNK activation and AP-1 and IL-2 reporter gene induction by costimulation of CD3 and CD28 during T-cell activation. Interestingly, we found that JNK activation in T-cells by TPA and Ca**2** ionophore was not inhibited by MEKK2 mutants. A similar result was obtained by Schaefer et al. (36), who showed that expression of a similar murine MEKK2 dominant negative mutant could not inhibit JNK activation by TPA and ionomycin in murine T-cells. Therefore, these results indicated that MEKK2 is not involved in transducing TPA- and Ca**2** ionophore-mediated T-cell-activating signals but was required for transducing TCR/CD3-mediated activating signals.

Recently we demonstrated that MEKK2 is a major upstream activator of the JNK MAPK through forming a triple molecular complex with JNK2/MEKK7 and JNK1 (28). Biochemical analysis of both human MEKK2 (28) and murine MEKK2 (18, 41) showed that they are potent MAPKKKs for the JNK MAPK module. Murine MEKK2 is also able to activate the Erk and p38 MAPKs in addition to JNK MAPKs (18, 36, 41). However, we found that human MEKK2 activated JNK but not Erk in Jurkat T-cells. Because stimulation of T-cells can activate both the JNK and Erk pathways, our results suggested that MEKK2 might be involved in transducing TCR/CD3 signals to the JNK cascade but not to the Erk cascade.

How MEKK2 is involved in TCR/CD3 signal transduction in T-cells is still unknown. Schaefer et al. (36) showed that murine MEKK2 is rapidly translocated to the T-cell-antigen-presenting cell interface in response to antigen stimulation, suggesting that participation of MEKK2 in TCR/CD3 was required for signaling to downstream targets. In our study, we found that stimulation of CD3 in T-cells also resulted in human MEKK2 tyrosine phosphorylation, suggesting that MEKK2 is a target of the TCR/CD3-associated protein tyrosine kinases. It is possible that MEKK2 tyrosine phosphorylation after TCR stimulation is involved in regulating MEKK2 activity. Alternatively, tyrosine phosphorylation of MEKK2 may provide additional docking sites for MEKK2 to interact with TCR/CD3. Further studies on this issue should increase our understanding of the general mechanism of MEKK2 activation.

Finally, transient expression of full-length MEKK2 in T-cells was able to activate JNK constitutively in the absence of stimulation, but no endogenous JNK activation was observed in unstimulated Jurkat T-cells (Fig. 2A). This was not due to the absence of MEKK2 in Jurkat T-cells, because by using anti-serum raised against the N terminus and C terminus, respectively, we easily detected MEKK2 protein in Jurkat T-cells (Fig. 1C). Therefore, the endogenous MEKK2 in T-cells must be inactive before stimulation. It is possible that MEKK2 is tightly regulated by inhibitors or by compartmentalization, and such regulation could be disrupted in transient transfection assay. In this regard, we recently isolated an MEKK2-associated protein, and analysis of it will shed light on this issue.

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