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TNF-Mediated Activation of the Stress-Activated Protein Kinase Pathway: TNF Receptor-Associated Factor 2 Recruits and Activates Germinal Center Kinase Related

Chong-Shan Shi,1* Antonio Leonardi,1* John Kyriakis,† Ulrich Siebenlist,§ and John H. Kehrl2*

TNF-induced activation of stress activated protein kinases (SAPKs, Jun NH2-terminal kinases) requires TNF receptor associated factor 2 (TRAF2). TRAF2 is a potent activator of a 95-kDa serine/threonine kinase termed germinal center kinase related (GCKR), also referred to as KHS1, which signals activation of the SAPK pathway. Consistent with a role for GCKR in TNF-induced SAPK activation, a kinase-inactive mutant of GCKR is a dominant negative inhibitor of TRAF2-induced SAPK activation. Here we show that TRAF2 interacts with GCKR. This interaction depended upon the TRAF domain of TRAF2 and the C-terminal 150 aa of GCKR. The full activation of GCKR by TRAF2 required the TRAF2 RING finger domain. TNF treatment of a T cell line, Jurkat, increased both GCRK and SAPK activity and enhanced the coimmunoprecipitation of GCKR with TRAF2. Similar results were found with the B cell line HS-Sultan. These findings are consistent with a model whereby TNF signaling results in the recruitment and activation of GCKR by TRAF2, which leads to SAPK activation. The Journal of Immunology, 1999, 163: 3279–3285.


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3 Abbreviations used in this paper: TNFR1, TNF receptor type 1; TRADD, TNFR-associated death domain; TRAF, TNFR-associated factor; RIP, receptor interacting protein; NIK, NF-eB-inducing kinase; SAPK, stress-activated protein kinase; JNK, Jun NH2-terminal kinase; GCK, germinal center kinase; GLK, germinal center-like kinase; GCKR, GCK related; HA, hemagglutinin; MBP, myelin basic protein; PEST, proline glutamic acid serine threonine; ASK1, apoptosis signal-regulating kinase 1.

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† C.-S.S. and A.L. contributed equally to this work.
Materials and Methods

Cell lines, plasmids, and constructs

The 293T cell line was obtained from Dr. O. Witte (Los Angeles, CA) following permission from Dr. D. Baltimore (Pasadena, CA), HS-Sultan, and Jurkat cells were obtained from the American Tissue Culture Collection (Manassas, VA). RT-PCR was used to generate a 486-bp fragment of TRAF2 from Jurkat cell RNA (primers, TCIGGIGCTAGAAAGCGGAT and GTICTIAAT/CGIGGAA/GGTGIGA). This PCR fragment was used to obtain a full-length TRAF2 cDNA clone from a human activated T cell cDNA library, which was cloned into pMT2T. The construct, which contains the RING finger and zinc finger domains of TRAF2 (amino acids 1–225) was created by inserting a Nhel-XhoI fragment of TRAF2 in pMT2T. The sequence upstream of the Nhel site was replaced with a linker encoding the first 4 aa. The construct containing the RING finger domain of TRAF2 (amino acids 1–105) was generated by inserting an EcoRI-EcoI fragment in pMT2T. The construct containing the zinc finger domain of TRAF2 (amino acids 76–282) was generated by PCR. The dominant negative TRAF2 (amino acids 87–501) and the TRAF domain of TRAF2 (amino acids 271–501) constructs were created by PCR using pMT2T TRAF2 as template and cloned into pcR3.1. The hemagglutinin (HA)-tagged full-length GCKR and its different mutants (amino acids 1–691, 1–599, 1–493, 1–396, and 386–846) were obtained by PCR with the appropriated restriction sites incorporated into the primers and using GCKR cDNA clone as a template and pcDNA-HA as a vector. The HA-GCKR (amino acids 1–283) construct was generated by releasing a XbaI restriction fragment using an endogenous XbaI site in the GCKR cDNA and a XbaI site in the polylinker of the pcDNA-HA-GCKR construct. This fragment was subcloned into the XbaI site in pcDNA-HA.

Metabolic labeling and immunoprecipitation of different TRAF2 constructs

293 cells (3 × 10⁶) were transfected with expression vectors that direct the expression of TRAF2 and truncated versions of TRAF2. Two days later, the cell culture media was replaced with media that lacked cysteine and methionine, but contained [³⁵S]cysteine and [³⁵S]methionine (50 μCi of [³⁵S] in vitro cell-labeling mix per milliliter of media; Amersham, Arlington Heights, IL). One hour later, the labeled cells were washed with cold PBS and lysed at 4°C in 1 ml of 1% Triton X-100 lysis buffer containing 25 mM HEPES, pH 7.5, 150 mM NaCl, 10% glycerol, 1 mM EDTA, and protease inhibitors (Roche Molecular Biochemicals, Indianapolis, IN). Nuclear and cell debris was removed by centrifugation at 14,000 rpm for 10 min at 4°C.

Cell extracts were incubated for 2 h at 4°C with 5 μl of anti-TRAF2 antiserum, and the immunoprecipitates were collected with 20 μl of protein A-Sepharose. The TRAF2 polyclonal antiserum raised against a GST fusion protein that contained the RING, and a portion of the zinc fingers of TRAF2 (1–225) was used throughout these experiments. Beads were washed four times with lysis buffer, boiled in SDS sample buffer, and the supernatant were subjected to SDS-PAGE and autoradiography.

In vitro kinase assays, coimmunoprecipitation, and immunoblotting

293T cells were cotransfected by calcium phosphate/DNA precipitation with pcDNA-HA-GCKR (1 μg), pMT3-HA-SAPK-p46 (1 μg), and constructs that direct either wild-type TRAF2 or various truncation mutants (2 μg). Transfected DNA levels were equalized using empty plasmid. Thirty-six hours following the transfection of 293T cells, HA immunoprecipitates were subjected to similar in vitro kinase assays, and lysate were extracted in 1 ml of lysis buffer (20 mM Tris, pH 8.0, 137 mM NaCl, 2 mM EDTA, 1% Triton X-100, 1 mM sodium orthovanadate, 1 mM DTT, plus protease inhibitors) for 30 min on ice. The HA mAb (Babco, Richmond, CA) and the TRAF2 antiserum were used for the coimmunoprecipitation experiments in 293 cells. The TRAF2- and GCKR-specific antisera were used to examine the coimmunoprecipitation experiments in Jurkat and HS-Sultan cells (10 × 10⁶ cells). The immunoprecipitates were collected with the appropriate anti-Ig Ab coupled to magnetic beads (Dynabeads, Oslo, Norway). The beads were washed six times with lysis buffer. The bound proteins were eluted in SDS-sample buffer, fractionated by SDS-PAGE, and transferred to nitrocellulose using standard methodology. The signals were detected by enhanced chemiluminescence (Amershan, Arlington Heights, IL). For the coimmunoprecipitation following TNF signaling, 293T cells that had been transfected with HA-GCKR 2 days previously, HS-Sultan, or Jurkat cells were exposed to TNF (100 ng/ml, Endogen, Woburn, MA) for 15 min before cell lysis.

Results and Discussion

Considerable evidence indicates that the TNF-induced NF-κB and SAPK activation requires the recruitment of TRAF2 to the TNFR. Simple overexpression of TRAF2 leads to both NF-κB and SAPK activation. However, the TNF-induced signaling pathway that leads to NF-κB and SAPK activation diverges at the level of TRAF2. TRAF2-mediated NF-κB activation can be inhibited by a dominant negative form of NIK, and NIK overexpression leads to NF-κB activation, but not SAPK activation (11–13, 15–17). Conversely, TRAF2-mediated SAPK activation can be inhibited by a dominant negative form of GCKR, while NF-κB activation is unaltered. Furthermore, GCKR overexpression leads to NF-κB activation can be inhibited by a dominant negative form of NIK, and NIK overexpression leads to NF-κB activation, but not SAPK activation (11–13, 15–17). Conversely, TRAF2-mediated SAPK activation can be inhibited by a dominant negative form of GCKR, while NF-κB activation is unaltered. Furthermore, GCKR overexpression leads to NF-κB activation can be inhibited by a dominant negative form of NIK, and NIK overexpression leads to NF-κB activation, but not SAPK activation (11–13, 15–17). Conversely, TRAF2-mediated SAPK activation can be inhibited by a dominant negative form of GCKR, while NF-κB activation is unaltered. Furthermore, GCKR overexpression leads to NF-κB activation can be inhibited by a dominant negative form of NIK, and NIK overexpression leads to NF-κB activation, but not SAPK activation (11–13, 15–17). Conversely, TRAF2-mediated SAPK activation can be inhibited by a dominant negative form of GCKR, while NF-κB activation is unaltered. Furthermore, GCKR overexpression leads to NF-κB activation can be inhibited by a dominant negative form of NIK, and NIK overexpression leads to NF-κB activation, but not SAPK activation (11–13, 15–17). Conversely, TRAF2-mediated SAPK activation can be inhibited by a dominant negative form of GCKR, while NF-κB activation is unaltered. Furthermore, GCKR overexpression leads to NF-κB activation can be inhibited by a dominant negative form of NIK, and NIK overexpression leads to NF-κB activation, but not SAPK activation (11–13, 15–17). Conversely, TRAF2-mediated SAPK activation can be inhibited by a dominant negative form of GCKR, while NF-κB activation is unaltered. Furthermore, GCKR overexpression leads to NF-κB activation can be inhibited by a dominant negative form of NIK, and NIK overexpression leads to NF-κB activation, but not SAPK activation (11–13, 15–17). Conversely, TRAF2-mediated SAPK activation can be inhibited by a dominant negative form of GCKR, while NF-κB activation is unaltered. Furthermore, GCKR overexpression leads to NF-κB activation can be inhibited by a dominant negative form of NIK, and NIK overexpression leads to NF-κB activation, but not SAPK activation (11–13, 15–17). Conversely, TRAF2-mediated SAPK activation can be inhibited by a dominant negative form of GCKR, while NF-κB activation is unaltered.
whether they coimmunoprecipitated. For the TRAF2 immunoprecipitations and immunoblotting, we used a rabbit anti-TRAF2 antiserum raised against the RING finger and a portion of the zinc finger region. We found that the HA-GCKR immunoprecipitates contained TRAF2 and TRAF2 immunoprecipitates contained HA-GCKR (Fig. 1). We were unable to verify the presence of TRAF2 in the TRAF2 immunoprecipitates because the TRAF2 band merged with the H chain of Ig.

To map the site of interaction of TRAF2 with GCKR, we made a series of constructs that direct the expression of TRAF2 deletion mutants. First, we determined whether the truncated proteins were expressed and that the TRAF2 antiserum immunoprecipitated them. Cell lysates were prepared from 293T cells that had been in vivo labeled with $^{35}$S methionine and $^{35}$S cysteine following transfection with constructs that directed expression of TRAF2, TRAF2 (1–225), TRAF2 (1–105), and TRAF2 (98–501). TRAF2 immunoprecipitates were collected and analyzed by SDS-PAGE and autoradiography (Fig. 2). A strong band for each of the mutant proteins was observed, confirming that they were expressed and recognized by the TRAF2 antiserum. Next, we coexpressed HA-GCKR along with the TRAF2 or the truncated forms of TRAF2 in 293T cells and prepared HA immunoprecipitates. Neither TRAF2 (1–225), TRAF2 (1–105), or TRAF2 (76–282) coimmunoprecipitated with GCKR. However, TRAF2 (96–501), which lacks the TRAF2 RING finger and behaves as a dominant negative for TNF-induced SAPK and GCKR activation, readily coimmunoprecipitated with GCKR (Fig. 3, left). This suggests that the TRAF2/GCKR interaction depend upon the presence of the TRAF domain in TRAF2. In contrast, the TRAF1 protein, which fails to activate the SAPK pathway, did not coimmunoprecipitate with GCKR (C.-S.S., unpublished observations).

We also analyzed the various TRAF2 mutant proteins for their ability to activate GCKR and the SAPK pathway. GCKR activation was assessed by an in vitro kinase assay, which measures the ability of immunoprecipitated epitope-tagged GCKR to phosphorylate MBP (25). Similarly, SAPK activation was assayed by examining the ability of SAPK to phosphorylate a recombinant N-terminal fragment of c-Jun (18, 19). 293T cells were transfected with constructs that direct the expression of wild-type TRAF2 and

![FIGURE 2. The TRAF2 antisera immunoprecipitates TRAF2 and truncated forms of TRAF2. 293 cells were transfected with constructs that direct the expression of TRAF2, TRAF2 (98–501), TRAF2 (76–282), TRAF2 (1–105), and TRAF2 (1–225). Two days following the transfection, cell lysates from metabolically labeled cells were prepared and subjected to immunoprecipitation with the TRAF2 antisera. The immunoprecipitates were size-fractionated by SDS-PAGE, and the dried gel was autoradiographed. Exposure is overnight.](http://www.jimmunol.org/)

![FIGURE 3. The TRAF domain of TRAF2 is required for association with GCKR, but the RING finger is needed for GCKR activation. 293T or 293 cells were transfected with HA-GCKR (2 μg) and either 2 μg of TRAF2 (1) or various TRAF2 mutant constructs (2–5). TRAF2 immunoprecipitates (TRAF2 I.P.) were examined for the presence of HA-GCKR by HA-immunoblotting (lanes 1–5, left). Anti-HA immunoprecipitates were subjected to an in vitro kinase assay using MBP as a substrate to assess GCKR activation. The fold inductions compared with GCKR alone are shown and are a mean of five experiments; a representative autoradiograph is shown (lane 1, GCKR alone; lanes 2–6, GCKR with TRAF2 or TRAF2 mutants). 293 or 293T cells were also transfected with HA-SAPK (1 μg) and a control plasmid, TRAF2, or the various TRAF2 mutant constructs (2 μg). Anti-HA immunoprecipitates were subjected to an in vitro kinase assay using c-Jun (79) as a substrate to assess SAPK activation. The fold inductions compared with a control plasmid alone are shown and are a mean of three experiments; a representative autoradiograph is shown (lane 1, control plasmid alone; lanes 2–6, TRAF2 or TRAF2 mutants). The expression of TRAF2 and TRAF2 deletion mutants, HA-GCKR and HA-SAPK, were verified by immunoblotting. Reblotting the anti-TRAF2 immunoprecipitates verified the presence of the TRAF2 (76–282), TRAF2 (1–105), and TRAF2 (1–225) (data not shown).](http://www.jimmunol.org/)
the truncated version. All of the TRAF2 mutant proteins including TRAF2 (98–501) had significantly impaired abilities to activate GCKR, although the constructs that expressed either the RING or zinc finger domains had low levels of activity in 293T cells (Fig. 3, right). The small increases in the basal GCKR activity noted following expression of the TRAF2-truncated proteins were not observed when a similar experiment was performed in 293 cells, suggesting that it results from the high expression levels obtained in 293T cells (C.-S.S. unpublished observations). Nevertheless, these small increases in GCKR activity were insufficient to result in SAPK activation in vivo as all the mutants were markedly impaired in their ability to activate SAPK. Only the wild-type TRAF2 significantly increased both GCKR and SAPK activity levels.

A prediction of our model that GCKR activation occurs downstream of TRAF2 activation is that the TRAF2 dominant negative mutant, which impairs TNF-α-induced SAPK activation (25), should not inhibit GCKR-induced SAPK activation. To verify this prediction, we cotransfected constructs that direct the expression of GCKR, the TRAF2 dominant negative mutant as well as the other TRAF2 mutants, and HA-SAPK. We found that neither TRAF2 (98–501) nor the other TRAF2 mutant proteins significantly impaired GCKR-induced SAPK activation (Fig. 4). We verified that SAPK, GCKR, and the TRAF2 mutant proteins were appropriately expressed by immunoblotting. Thus, GCKR acts downstream of TRAF2 in TNF-induced SAPK activation.

GCKR has an N-terminal catalytic domain and a large C-terminal regulatory domain, which has several proline-rich regions, one of which behaves as a CrkL interaction site. The C-terminal portion of GCK (amino acids 679–819) and a region between amino acids 270 and 329, which spans a PEST sequence, were both required for binding of TRAF2 to the GCK (27). To map the site of interaction of GCKR with TRAF2, we coexpressed various C-terminal-truncated HA-tagged GCKR proteins with wild-type TRAF2 and examined TRAF2 immunoprecipitates for the presence of HA-tagged GCKR proteins. While we readily detected full-length GCKR, we did not detect any of the truncation mutants despite adequate expression levels (Fig. 5). Thus, similar to GCK the C-terminal portion of GCKR is required for the interaction with TRAF2. The GCKR C-terminal region is well conserved with the corresponding regions in GLK and GCK (~60% identity between GCKR, GCK, and GLK over the C-terminal 110 aa), suggesting that all three kinases may use this region to interact with TRAF2 (Fig. 6).

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FIGURE 4. TRAF2 mutants do not inhibit GCKR-mediated SAPK activation. 293T cells were transfected with constructs that direct expression of GCKR, HA-SAPK, TRAF2 (98–501), TRAF2 (1–225), TRAF2 (1–105), and TRAF2 (76–282). HA-SAPK immunoprecipitates were subjected to an in vitro kinase using c-Jun (79) as a substrate. The levels of GCKR expression were detected with the GCKR-specific antiserum, the HA antisera was used to detect SAPK expression, and the TRAF2 antiserum was used to detect the TRAF2 mutants.

FIGURE 5. The C-terminal 150 aa of GCKR are required for the association of GCKR with TRAF2. HA-GCKR and HA-GCKR truncation mutants were expressed in 293T cells along with TRAF2. TRAF2 immunoprecipitations and cell lysates were examined for the presence of HA-GCKR (1) and the HA-GCKR truncation mutants (2–5) by anti-HA immunoblotting. The expression of TRAF2 was verified by immunoblotting cell lysates (not shown).
To examine whether the TRAF domain of TRAF2 and the regulatory domain of GCKR were sufficient to observe an interaction with GCKR, we coexpressed an N-terminal truncation of GCKR, HA-GCKR (386–846), with TRAF2 (272–501) in 293T cells. This truncated version of GCKR lacks the region corresponding to the first PEST-like sequence in GCK, which was necessary for GCK and TRAF2 to interact. We observed that HA-GCKR (386–846) strongly bound TRAF2 (272–501), as each protein readily coimmunoprecipitated with the other (Fig. 7A). Similar to the other TRAF2 mutants, TRAF2 (272–501) did not appreciably activate either GCKR or SAPK (data not shown). Thus, the first PEST-like sequence in GCKR is not necessary for its interaction with the TRAF domain of TRAF2.

Next, we sought to determine whether TNF signaling altered the interaction between TRAF2 and GCKR. We expressed HA-GCKR in 293T cells and stimulated the cells with TNF or not, immunoprecipitated the endogenous TRAF2, and examined the immunoprecipitates for the presence of HA-GCKR. TNF treatment induced in a significant increase in the amount of GCKR coimmunoprecipitating with TRAF2 (Fig. 7B). Attempts to visualize the recruitment of endogenous GCKR to endogenous TRAF2 in 293T cells were unsuccessful. We suspect this was because of the relatively low levels of GCKR in these cells.

Because GCKR is well expressed in both T and B lymphocytes, we sought to determine whether TNF exposure activated GCKR in lymphocyte cell lines. We stimulated Jurkat and HS-Sultan cells

FIGURE 6. The C-terminal region of GCKR is homologous to that of GCK and GLK. The C-terminal 152 aa of GCKR were aligned with the C-terminal 150 and 151 aa of GLK and GCK, respectively. The alignment was performed with the CLUSTALW program. Identical amino acids are indicated in gray.

FIGURE 7. The TRAF domain of TRAF2 interacts with the regulatory domain of GCKR, and TNF-α stimulation recruits GCKR to TRAF2. A, The coimmunoprecipitation of the TRAF domain of TRAF2 (T2 272–501) with GCKR (386–846). 293T cells were transfected with TRAF2 (272–501) and HA-GCKR (386–846). Two days later, cell lysates were prepared and immunoprecipitated with a HA mAb, a TRAF2 polyclonal antisera, or a control Ab. The cell lysate (lane 1) and immunoprecipitates (lanes 2 and 3) were analyzed by immunoblotting with anti-HA or anti-TRAF2 Abs as indicated. HA-GCKR (386–846) and T2 (272–501) are indicated with arrows. B, TNF signaling recruits GCKR to TRAF2. 293T cells transfected with HA-GCKR were stimulated with media or TNF-α (100 ng/ml) for 15 min before lysis. Following transfer to nitrocellulose, the TRAF2 immunoprecipitates (lanes 1 and 2) and the cell lysate (lane 3) were analyzed by immunoblotting the upper portion of the membrane with anti-HA and the lower portion of the membrane with anti-TRAF2. The photograph is of a composite of the two blots joined at approximately the 68-kDa marker. HA-GCKR and TRAF2 are indicated by arrows.
A.

Jurkat
HS-Sultan

- 4.2 3.8 3.6
- 8.1 7.5 5.2 Fold Increase

- 1.3 3.3 2.8
- 1.8 3.8 4.4 Fold Increase

GCKR
SAPK

MBP and GST-Jun (79)

GCKR and SAPK activation and the association of GCKR with TRAF2. A, TNF triggers GCKR and SAPK activation. Jurkat and HS-Sultan cells were stimulated with TNF-α (100 ng/ml) for the indicated times. GCKR and SAPK immunoprecipitates were subjected to in vitro kinase assays using MBP and GST-Jun (79) as substrates. The intensities of the bands were assessed by autoradiography and analyzed by NIH Image (Bethesda, MD). The results are reported as fold increase compared with nonstimulated control cells. The levels of GCKR and SAPK are shown below and were detected by immunoblotting with GCKR- and SAPK-specific Abs. B, TNF-α stimulation triggers an association between GCKR and TRAF2. Jurkat and HS-Sultan cells were stimulated with TNF-α for 15 min (lanes 2, 3, 5, and 6) or not (lanes 4 and 7). TRAF2 or preimmune immunoprecipitates were fractionated by SDS-PAGE and immunoblotted for GCKR. GCKR levels in the cell lysates are shown (lane 1 and 8).

B.

Jurkat
HS-Sultan

- GCKR

- TNF-α

- Lyse
- Preimmune
- cTRAF2
- cTRAF2

- Fold Increase

FIGURE 8. TNF stimulation of Jurkat and HS-Sultan cells results in GCKR and SAPK activation and the association of GCKR with TRAF2. A, TNF triggers GCKR and SAPK activation. Jurkat and HS-Sultan cells were stimulated with TNF-α (100 ng/ml) for the indicated times. GCKR and SAPK immunoprecipitates were subjected to in vitro kinase assays using MBP and GST-Jun (79) as substrates. The intensities of the bands were assessed by autoradiography and analyzed by NIH Image (Bethesda, MD). The results are reported as fold increase compared with nonstimulated control cells. The levels of GCKR and SAPK are shown below and were detected by immunoblotting with GCKR- and SAPK-specific Abs. B, TNF-α stimulation triggers an association between GCKR and TRAF2. Jurkat and HS-Sultan cells were stimulated with TNF-α for 15 min (lanes 2, 3, 5, and 6) or not (lanes 4 and 7). TRAF2 or preimmune immunoprecipitates were fractionated by SDS-PAGE and immunoblotted for GCKR. GCKR levels in the cell lysates are shown (lane 1 and 8).

with TNF, immunoprecipitated GCKR and SAPK with specific antisera, and subjected the immunoprecipitates to in vitro kinase assays using MBP or c-Jun 1–79, respectively (Fig. 8). We found that GCKR was rapidly activated in both Jurkat and HS-Sultan following TNF exposure. TNF also induced SAPK activation in these two cell lines, although it was slightly delayed compared with GCKR activation. Next, we determined whether we could detect endogenous GCKR associated with endogenous TRAF2 following TNF signaling. We found low levels of GCKR immunoprecipitated with TRAF2 before TNF stimulation; however, following TNF stimulation we detected a marked increase in the amount of GCKR communoprecipitating with TRAF2.

We conclude from these experiments that TNF stimulation results in the recruitment and activation of GCKR by TRAF2, which leads to SAPK activation. The TRAF domain of TRAF2 is required for the recruitment of GCKR. However, the interaction of the TRAF domain with GCKR is insufficient for high-level GCKR activation for which the TRAF2 RING and perhaps the zinc fingers are required. How the RING and zinc fingers of TRAF2 contribute to GCKR activation is unclear. They may alter the conformation of GCKR triggering GCKR autophosphorylation or perhaps they induce the dissociation of an inhibitor. Alternatively, they may be necessary for interaction with other proteins that have a role in GCKR activation. It will be of interest to determine whether GCKR and NIK compete for the same interaction site on TRAF2 and, in particular, whether the WKI motif defined in TRAF2 (16), which is important for its interaction with NIK, is required for the TRAF2-GCKR interaction. However, suggesting that GCKR and NIK may interact with separate sites on TRAF2, the overexpression of NIK failed to interfere with the communoprecipitation of GCKR with TRAF2, and, conversely, the overexpression of GCKR failed to inhibit the communoprecipitation of NIK with TRAF2 (C.-S.S., unpublished observations).

Overall, our findings are consistent with the previously described bifurcation of TNF-induced NF-κB and SAPK activation at the level of TRAF2 and support the model that TRAF2 functions as a docking protein for additional signaling molecules that trigger nonredundant signaling cascades (16, 17). Here, we have shown that GCKR and likely by analogy GCK and GLK function as a downstream effector of TRAF2 to trigger the SAPK pathway. Why these highly related kinases should all link TRAF2 to the SAPK pathway remains to be determined, although with further study variations in tissue distribution and subtle differences in their regulation are likely to emerge. In addition, how the GCK family of kinases and ASK1, which has also been shown to have a role in TNF-induced SAPK activation, interface remains to be determined (28). Like GCKR ASK1 is TNF-inducible and recruited to the TNF-receptor following TNF-signaling. The C-terminal portion of ASK1 is necessary for the interaction of ASK1 and TRAF2 (28), but it does not share a significant degree of homology with the C-terminal region of GCK, GCKR, and GLK (C.-S.S., unpublished observations). A catalytically inactive form of either GCKR or ASK1 impairs TNF- and TRAF2-induced SAPK activation in 293 cells. However, GCKR-induced SAPK activation is not impaired by the expression of the ASK1 mutant protein, indicating that ASK1 is unlikely downstream from GCKR in the TNF-induced SAPK activation (25). Targeted inactivation of the various GCK family members as well as ASK1 in mice will likely be required to formally evaluate the relative importance of these kinases in TNF-induced SAPK activation in vivo.

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References


