MIP-T3, a Novel Protein Linking Tumor Necrosis Factor Receptor-associated Factor 3 to the Microtubule Network*

Received for publication, February 9, 2000, and in revised form, April 28, 2000
Published, JBC Papers in Press, May 1, 2000, DOI 10.1074/jbc.M001095200

Lei Ling† and David V. Goeddel
From Tularik Inc., South San Francisco, California 94080

In this study, we report the identification of a novel tumor necrosis factor receptor-associated factor 3 (TRAF3)-interacting protein designated MIP-T3. MIP-T3 is a 83-kDa protein with no significant homology to known mammalian proteins. MIP-T3 mRNA and TRAF3 mRNA are ubiquitously expressed, and TRAF3 is the only TRAF protein to interact with MIP-T3. The MIP-T3-TRAF3 interaction requires the coiled-coil TRAF-N domain of TRAF3. To our knowledge, this is the first case of a TRAF-binding protein that interacts with a single member of the TRAF family specifically through a TRAF-N coiled-coil domain. MIP-T3 binds to Taxol-stabilized microtubules and to tubulin in vitro, and MIP-T3 recruits TRAF3 to microtubules when both proteins are overexpressed in HeLa cells. In a 293 cell line stably expressing CD40, TRAF3 is released from the TRAF3-MIP-T3 complex and recruited to the CD40 receptor upon CD40 ligand stimulation. MIP-T3 may provide a novel mechanism in sequestering TRAF3 to the cytoskeletal network.

Several members of the TRAF family, including TRAF2, TRAF5, and TRAF6, have been implicated in various signal transduction pathways leading to the activation of the transcription factor nuclear factor-κB (NF-κB) (9, 10, 12, 13, 29, 32). Overexpression of TRAF2, TRAF5, or TRAF6 activates NF-κB, and truncated versions of TRAF2, TRAF5, and TRAF6 lacking zinc binding domains act as dominant negative inhibitors of NF-κB activation mediated by various receptors, suggesting that these TRAFs are common mediators for NF-κB activation (9, 10, 12, 13, 29, 32). On the other hand, TRAF1, TRAF3, and TRAF4 do not activate NF-κB when overexpressed (16). TRAF2, TRAF5, and TRAF6 also activate c-Jun N-terminal kinase when overexpressed (16, 17).

Additional data on the physiological roles of four TRAF family members have been determined by gene targeting experiments in mice (18–23). TRAF2–/– (19), TRAF3–/– (21), and TRAF6-deficient (23) mice appear normal at birth but become progressively runted and die prematurely. TRAF2-deficient mice have elevated levels of serum TNF. Ex vivo assays demonstrated that TRAF2–/– embryonic fibroblasts are highly sensitive to TNF-induced cell death and that in the absence of TRAF2, TNF-mediated c-Jun N-terminal kinase activation is greatly reduced. TRAF2 deficiency results in partial inhibition of TNF-induced NF-κB activation, and complete inhibition of CD40-induced NF-κB activation (18, 19). Recent studies on TRAF6–/– mice revealed that TRAF6 is required for interleukin 1, CD40, and lipopolysaccharide-induced NF-κB activation (23). Moreover, TRAF6-deficient mice are osteopetrotic, with defects in bone remodeling and tooth eruption due to impaired osteoclast function (23). Unlike TRAF2-, TRAF3-, and TRAF6-deficient mice, TRAF5–/– mice are healthy through 24 weeks of age, but the CD40 and CD27 signaling pathways are impaired in these mice (22). Collectively, these results suggested that TRAF2, TRAF5, and TRAF6 could act redundantly or specifically in particular signaling cascades.

TRAF3 was originally identified as a molecule that binds the cytoplasmic domains of the TNFR family member CD40 and the Epstein-Barr virus latent membrane protein LMP1 (4–7). Subsequently, it has been shown to interact with the cytoplasmic tails of the TNFR family members lymphotoxin β-receptor (LTβR), CD27, CD30, OX40, HEPES virus entry mediator, and receptor activator of NF-κB (24–32). The interaction of TRAF3 with both CD40 and LTβR has been shown to be ligand-dependent and to occur in nontransfected cells (33, 34). However, deletion of TRAF3 by gene targeting does not seem to affect either CD40-induced B cell proliferation or CD23 up-regulation in mice (21), nor do TRAF3-deficient mice have defective lymph node genesis as seen in LTβR-knockout mice (21). However, reconstitution of mice with TRAF3–/– fetal liver cells revealed a requirement for TRAF3 in T cell-dependent immune responses (21).

To gain more insights about the physiological roles of TRAF3, we performed yeast two-hybrid cloning to search for
TRAF3-interacting proteins. We identified a novel protein, MIP-T3, that is endogenously associated with TRAF3 and that appears to provide a link between TRAF3 and the microtubule network.

**EXPERIMENTAL PROCEDURES**

Reagents—Taxol, nucodazole, anti-flag monoclonal antibody M2, anti-tubulin, and FITC- or rhodamine-conjugated secondary antibodies were purchased from Sigma. Anti-Myc, anti-Flag, anti-TRAF3, and anti-CD40 antibodies were from Santa Cruz Biotechnology. The rabbit anti-MIP-T3 antisera was raised against a 35-mer peptide, KKILETKKYDKYELQSSPKQGEKERSLFESAWKKEK, and was affinity-purified (Covance).

Expression Vectors—Mammalian expression vectors encoding TRAF3's 1–6 have been described (16). MIP-T3 was expressed as N-terminally tagged Flag or Myc fusion proteins using the pRK vector. Deletion mutants of MIP-T3 and TRAF3 were generated by polymerase chain reaction.

**RESULTS**

Isolation of cDNA Clones Encoding TRAF3-Interacting Proteins—To increase our understanding of the function of TRAF3, we performed yeast two-hybrid interaction cloning to screen for proteins that associate directly with TRAF3. An expression vector in which full-length TRAF3 was fused to the LexA DNA binding domain was used as a bait to screen a HeLa cDNA library (36). From approximately 10 million transformants, 10 independent positive clones were obtained, as determined by activation of *Leu* and *LacZ* reporter genes. One of these clones encodes I-TRAF/TANK, a protein previously shown to be associated with various TRAFs (37, 38). Three of these clones were independent isolates encoding portions of a novel protein. The longest insert from these three clones contains an open reading frame encoding 302 amino acids. The interaction between TRAF3 and this 302 amino acid protein was verified by retransformation into yeast cells (data not shown).

We screened a HeLa λZap cDNA library using the partial cDNA as probe. DNA sequence analysis of the positive clones revealed an open reading frame predicted to encode a protein of 625 amino acids that we have designated MIP-T3, for microtubule-interacting protein that associates with TRAF3 (Fig. 1). Data base searches utilizing the BLAST and FASTA programs failed to identify any mammalian proteins having significant sequence similarity to MIP-T3. However, a *Caenorhabditis elegans* protein (*GenBank* accession number U49945) of unknown function shares an overall 22% identity and 28% similarity to MIP-T3. The C-terminal 100 amino acids of MIP-T3 form a coiled-coil domain (aa 525–625).

Northern blot analysis indicated that MIP-T3 mRNA was expressed in all human tissues examined (Fig. 2A). This result is consistent with MIP-T3 involvement in TRAF3 signal transduction, as TRAF3 is also expressed ubiquitously (6, 39). Two different sizes (4.4 and 2.4 kb) of MIP-T3 transcripts were visualized in most tissues on the Northern blot. The two transcripts encode the same open reading frame with the longer transcript having an extra 2 kb of 3′ untranslated region, as revealed from the sequences of MIP-T3 clones from the HeLa λZap cDNA library. In addition, testis contains an additional MIP-T3 transcript of 2.7 kb.

Polyclonal antibody against MIP-T3 was generated by immunizing rabbits with a 35-mer peptide, KKILETKKYDKYELQSSPKQGEKERSLFESAWKKEK, derived from amino acids...
476–510 of MIP-T3. Rabbit anti-MIP-T3 antiserum specifically recognized a protein of approximately 83 kDa in nontransfected 293, HeLa, and SW480 cells by immunoprecipitation followed by immunoblotting with the same antibody (Fig. 2B), which is the same size as the MIP-T3 expressed in transiently transfected 293 cells.

**MIP-T3 Specifically Interacts with TRAF3 in Vitro and in Vivo**—We performed *in vitro* GST pull-down assays to confirm the interaction between MIP-T3 and TRAF3 observed in the two-hybrid system. All six TRAFs were 35S-labeled by *in vitro* translation using SP6 RNA polymerase. Equal amounts of TRAFs were incubated with GST or GST-MIP-T3 (aa 324–625) protein immobilized on glutathione beads. As shown in Fig. 3A, MIP-T3 strongly associated with TRAF3 but not with TRAF1, TRAF2, TRAF4, or TRAF5. MIP-T3 also very weakly associated with TRAF6. The interaction of MIP-T3 with TRAF3 was further analyzed in mammalian cell coimmunoprecipitation assays. The full-length MIP-T3 construct containing an N-terminal Myc epitope tag was transiently coexpressed in 293 cells with Flag epitope-tagged TRAFs. Cell lysates were immunoprecipitated using a monoclonal antibody against the Flag epitope or control mouse IgG, and coprecipitating MIP-T3 was detected by immunoblotting analyses with anti-Myc polyclonal antibody (Fig. 3B). In this assay, MIP-T3 specifically coprecipitates TRAF3 but not TRAF1, TRAF2, TRAF4, TRAF5, or TRAF6, consistent with the results of the *in vitro* GST pull-down assay.

**The Coiled-coil Regions in TRAF3 and MIP-T3 Contribute to Their Interaction**—TRAF3 contains a N-terminal ring finger domain followed by several zinc fingers (4, 38). The C-terminal half of TRAF3 is the TRAF domain, which is conserved among all six members of the TRAF family. The TRAF domain of TRAF3 can be further subdivided into the TRAF-N (aa 264–415) and TRAF-C (aa 416–568) domains (12, 38). The TRAF-N domain of TRAF3 contains an extended coiled-coil region (4, 12, 38). To determine which regions of TRAF3 contribute to MIP-T3 binding, various 35S-labeled TRAF3 deletion mutants were assayed for association with a GST-MIP-T3 (aa 324–625) fusion protein (Fig. 4A). The region containing the coiled-coil TRAF-N domain of TRAF3 (aa 267–376) is sufficient for binding to MIP-T3. Similar results were observed in 293 cell cotransfection experiments (Fig. 4B). Only TRAF3 deletion mutants (aa 1–376 and 267–568) containing the coiled-coil TRAF-N region were able to retain MIP-T3 binding activity,
whereas TRAF3 deletion mutants (aa 1–112, 1–266, and 390–568) corresponding to other domains of TRAF3 were not able to bind MIP-T3. These binding results are summarized in Fig. 4C. Similarly, to determine which domains of MIP-T3 contribute to the interaction with TRAF3, various MIP-T3 deletion mutants were generated. In the GST pull-down assays, the coiled-coil region of MIP-T3 (aa 525–625) is sufficient for binding to TRAF3. 293 cells were transiently transfected with expression vectors encoding Myc epitope-tagged MIP-T3 (full-length) and the Flag-tagged TRAFs 1–6. After 36 h, extracts were prepared and immunoprecipitated (IP) with anti-Flag monoclonal antibody or control mouse IgG. Coprecipitating Myc-MIP-T3 was detected by immunoblotting (IB) analysis using the anti-Myc polyclonal antibody (top panel). The amount of TRAFs immunoprecipitated and the expression level of Myc-MIP-T3 in total cell extracts were determined by immunoblotting with anti-Flag polyclonal antibody (bottom panel). The positions of MIP-T3 and TRAFs are indicated.

MIP-T3 Links TRAF3 to the Microtubule Network

**Fig. 3.** Interaction of MIP-T3 with TRAF3 in vitro and in vivo. A, in vitro GST pull-down assay. 35S-Labeled TRAFs were incubated with immobilized GST or GST-MIP-T3 (aa 324–625) fusion proteins and processed as described under “Experimental Procedures.” Bound proteins were resolved by SDS-PAGE and analyzed by autoradiography. The amounts of in vitro translated TRAF proteins used in the reactions are shown in the bottom panel. B, in vivo interaction of MIP-T3 with TRAF3. 293 cells were transiently transfected with expression vectors encoding Myc epitope-tagged MIP-T3 (full-length) and the Flag-tagged TRAFs 1–6. After 36 h, extracts were prepared and immunoprecipitated (IP) with anti-Flag monoclonal antibody or control mouse IgG. Coprecipitating Myc-MIP-T3 was detected by immunoblotting (IB) analysis using the anti-Myc polyclonal antibody (top panel). The amounts of in vitro translated TRAF3 deletion mutant proteins used in the reactions are shown in the bottom panel. C, summary of association data from A and B.

**Fig. 4.** Interaction of TRAF3 deletion mutants with MIP-T3. A, in vitro GST pull-down assay. The various 35S-labeled TRAF3 deletion mutants were incubated with purified GST or GST-MIP-T3 (aa 324–625) bound to glutathione-Sepharose. Bound proteins were fractionated by 4–20% gradient SDS-PAGE and exposed to x-ray film. The amounts of in vitro translated TRAF3 deletion mutant proteins used in the reactions are shown in the bottom panel. B, in vivo interaction of MIP-T3 with TRAF3 deletion mutants. 293 cells were transiently transfected with the Myc epitope-tagged MIP-T3 (aa 324–625) and the Flag-tagged TRAF3 deletion mutants. After 36 h, extracts were prepared and immunoprecipitated (IP) with anti-Flag monoclonal antibody or control mouse IgG. Coprecipitating Myc-MIP-T3 was detected by immunoblotting (IB) analysis using the anti-Myc polyclonal antibody (top panel). The amounts of TRAF3 deletion mutants immunoprecipitated were determined by immunoblotting with anti-Flag polyclonal antibody (bottom panel). C, summary of association data from A and B.
Association of the MIP-T3/TRAF3 Complex with Microtubular Structures—
To gain insight into the functional importance of MIP-T3 and TRAF3, we studied their subcellular localization by immunofluorescence microscopy. HeLa cells were transiently transfected with Myc-tagged MIP-T3 or Flag-tagged TRAF3. These epitope tags enabled us to visualize the expressed proteins by immunofluorescence using the corresponding epitope-specific affinity-purified antibodies. 24 h after transfection, the cells were fixed, permeabilized, and then incubated with anti-Myc or anti-Flag primary antibodies followed by fluorescently labeled secondary antibodies. Counterstaining with DAPI was included to visualize the nucleus (data not shown). When expressed separately, both MIP-T3 and TRAF3 had a cytosolic localization (Fig. 7, A and B); however, when MIP-T3 and TRAF3 were co-expressed, both proteins localized to cytoskeletal structures (Fig. 7, C). Pretreating cells with 0.2% Triton before fixation (40) showed the same cytoskeleton localization results (data not shown). Several properties indicate that this cytoskeletal structure is very likely microtubules. First, treatment with Taxol (a microtubule stabilizing drug) leads to the formation of organized bundles around the nucleus (41). Second, treatment with nocodazole (a microtubule destabilizing drug) destroyed this structure (41) (Fig. 7, C). Indeed, simultaneously staining of these cells with anti-Myo5 and anti-tubulin antibodies revealed the co-localization of MIP-T3 and tubulin (Fig. 7D). Similarly, staining of these cells with anti-Flag (to visualize TRAF3) and anti-tubulin antibodies revealed the co-localization of TRAF3 and tubulin (data not shown). Therefore, MIP-T3 and TRAF3 are co-localized to microtubule structure when co-expressed.

MIP-T3 Links TRAF3 to the Microtubule Network

**FIG. 5.** Interaction of MIP-T3 deletion mutants with TRAF3. A, in vitro GST pull-down assay. The various 35S-labeled MIP-T3 deletion mutants were incubated with purified GST or GST/TRAF3 bound to glutathione-Sepharose beads. Bound proteins were fractionated by 4–20% gradient SDS-PAGE and exposed to x-ray film. The amounts of in vitro translated MIP-T3 deletion mutant proteins used in the reactions are shown in the bottom panel. B, in vivo interaction of MIP-T3 deletion mutants with TRAF3. 293 cells were transiently transfected with the Myc epitope-tagged MIP-T3 deletion mutants and the Flag-tagged TRAF3. After 36 h, extracts were prepared and immunoprecipitated (IP) with anti-Flag monoclonal antibody or control mouse IgG. Coprecipitating Myc-MIP-T3 was detected by immunoblotting (IB) with anti-Myc polyclonal antibody (top panel). The amount of TRAF3 immunoprecipitated and the expression level of Myc-MIP-T3 deletion mutants in total cell extracts were determined by immunoblotting with anti-Flag polyclonal antibody (middle panel) and anti-Myc polyclonal antibody (bottom panel). C, summary of association data from A and B.
Next, we co-expressed Myc-tagged MIP-T3 with six different Flag-tagged TRAF proteins in HeLa cells. The transfected cells were simultaneously stained with anti-Myc antibody to visualize MIP-T3 (Fig. 8) and with anti-Flag antibody to visualize co-transfected TRAFs. MIP-T3 localized to the microtubules only when co-expressed with TRAF3 (Fig. 8). Similarly, TRAF3 was the only TRAF protein to localize to microtubules when co-expressed with MIP-T3 (data not shown).

*MIP-T3 Binds to Taxol-stabilized Microtubules and to Tubulin—* To examine the ability of MIP-T3 to bind to microtubules in vitro, we utilized the MAP spin-down assay (43, 44). Taxol-stabilized microtubules were incubated with in vitro translated MIP-T3. The microtubule pellets were collected by centrifugation, washed, and analyzed for the presence of MIP-T3 by autoradiography (Fig. 9A). MIP-T3 was able to bind polymerized microtubules (Fig. 9A, lane 1), whereas no microtubule binding was observed for negative control BSA protein (data not shown). No MIP-T3 protein was pelleted in the absence of microtubules.

To determine whether the MIP-T3 protein contains a specific domain that might be responsible for microtubule binding, various MIP-T3 deletion mutants were used. These mutants...
were synthesized by in vitro translation and subjected to the MAP spin-down assay. As shown in Fig. 9A, deletion of C-terminal coiled-coil region of MIP-T3 does not affect the microtubule binding ability of MIP-T3. However, deletion of the N-terminal 250 amino acids significantly reduced the binding of MIP-T3 to microtubules. These results demonstrate that MIP-T3 utilizes different regions for binding to TRAF3 and microtubules.

Similarly, immobilized GST-MIP-T3 fusion proteins were incubated with purified tubulin. After extensive washing, the tubulin bound on GST fusion protein beads was detected by immunoblotting with anti-tubulin antibody (Fig. 9B). GST protein alone does not bind tubulin. GST-MIP-T3 (full-length) protein, as well as GST-MIP-T3 (aa 1–525) and GST-MIP-T3 (aa 1–425) fusion proteins bind tubulin efficiently. Deletion of the N-terminal 323 amino acids of MIP-T3 abolishes its tubulin binding ability, which is consistent with the MAP spin-down assay results. The microtubule binding properties of MIP-T3 are summarized in Fig. 9C.

CD40 Ligand Induces Dissociation of TRAF3 from the TRAF3-MIP-T3 Complex—To examine the effect of cytokines on the complex formation of MIP-T3 with TRAF3, we used a 293.CD40 cell line that stably expresses Flag-tagged CD40. Following stimulation with a membrane preparation of CD40 ligand for various times, 293.CD40 cells were lysed, and extracts were immunoprecipitated with an anti-MIP-T3 rabbit polyclonal antibody or with an anti-Flag monoclonal antibody. The coprecipitating TRAF3 was detected by immunoblotting with an anti-TRAF3 monoclonal antibody. These experiments showed that TRAF3 dissociates from the endogenous TRAF3-MIP-T3 complex and is recruited to the CD40 receptor complex following CD40 ligand stimulation in a time-dependent manner (Fig. 10).

**Discussion**

In this study, we report the identification of a novel TRAF3-interacting protein, MIP-T3. MIP-T3 is a 83-kDa protein with no significant homology to known mammalian proteins. Like TRAF3, MIP-T3 is also ubiquitously expressed. Interestingly, TRAF3 is the only TRAF protein that interacts with MIP-T3, and this interaction requires the coiled-coil TRAF-N domain of TRAF3. To our knowledge, this is the first case of a TRAF-binding protein that interacts with a single member of the TRAF family specifically through a coiled-coil TRAF-N domain. It has been shown that the coiled-coil domain of TRAF3 is also important for efficient recruitment of TRAF3 to the LTβR and for TRAF3 self-association (45). We evaluated the ability of MIP-T3 to activate NF-κB, a known TRAF-mediated pathway. Overexpression of MIP-T3 alone or in combination with TRAF3 failed to activate NF-κB (data not shown). This result is consistent with data showing that TRAF3 is not required for the activation of NF-κB-dependent pathways by the CD40 ligand in TRAF3-deficient mice (21).

An interesting feature of MIP-T3 was revealed by immunofluorescence microscopy studies. MIP-T3 and TRAF3 are cytosolic proteins when expressed separately. However, when MIP-T3 and TRAF3 are co-expressed, they both localize to microtubular structures (Figs. 7 and 8). Moreover, MIP-T3 binds to Taxol-stabilized microtubules and to tubulin in vitro. It can be postulated that the interaction between MIP-T3 and TRAF3 further facilitates the microtubule binding ability of...
MIP-T3 Links TRAF3 to the Microtubule Network

MIP-T3. We observed that when cells express a high level of MIP-T3 (for example, when high levels of DNA are transfected or when cells are stained 40 h after transfection), MIP-T3 itself can localize to microtubule even in the absence of TRAF3 (data not shown). When MIP-T3 is expressed at low levels, it is mainly found in the cytosol, with co-expression of TRAF3 being required to bring both proteins to microtubules. However, it remains to be investigated whether co-expression of MIP-T3 and TRAF3 increases the stability of microtubules. It would also be interesting to determine the microtubule binding domain of MIP-T3. It was identified in the N-terminal region, between amino acids 51 and 250. This region is highly charged and rich in lysine and glutamic acid residues. The predominance of lysine and glutamic acid residues is also a typical feature of the tubulin binding region in MAP-1B, which contains reiterated KKE motifs (49). These motifs appear to be important for the interaction of MAP-1B with microtubules (49). Because there is a KKE motif around amino acid 243 of MIP-T3, it seems possible that the interaction of MIP-T3 with tubulin may occur by a similar mechanism.

Interestingly, the MIP-T3/TRAF3 complex exists in non-transfected cells. This complex can be dissociated upon CD40 ligand stimulation in 293.CD40 cells, whereas TRAF3 is recruited to the CD40 receptor. The sequestering of TRAF3 by MIP-T3 is reminiscent of the regulation of Smads (50). In transforming growth factor β pathway, microtubules serve as a cytoplasmic sequestering network for Smads in unstimulated cells. Transforming growth factor β triggers dissociation of Smads from microtubule, phosphorylation, and nuclear localization of Smad2 and Smad3, with consequent activation of transcription inside the nucleus (50).

Our experiments provide in vitro and in vivo evidence for TRAF3-MIP-T3/microtubule association and evidence that TRAF3-MIP-T3 association can be further regulated by activation of cytokine receptors such as CD40. It has been demonstrated that tubulin and microtubules are capable of influencing cellular signaling through direct interaction with a variety of signaling molecules (51–54). Therefore, it is possible that through binding to MIP-T3, tubulin or microtubules may regulate the cellular localization and/or functions of MIP-T3 and TRAF3. Additionally, tubulin is known to associate with a variety of cellular membranes, including the plasma membrane, endosomes, microsomes, and various organelles (51). Thus, the potential exists that microtubules could be responsible for directing MIP-T3 and TRAF3 to defined membrane microdomains in the cell. Finally, it is possible that such interactions may be dynamically regulated by stimuli in coordination with dynamic functions of the microtubule cytoskeleton during processes such as cell growth and differentiation.

In summary, we have identified a novel protein, MIP-T3, that interacts with TRAF3 and with microtubular structures. CD40 ligand stimulation induces the dissociation of the TRAF3-MIP-T3 complex. This signaling cascade could lead to the reorganization of microtubular cytoskeleton network, which direct cells for adhesion, movement, secretion, and other responses. Further investigations will be necessary to identify the precise roles of MIP-T3 during its association with TRAF3 and microtubules in cytokine signaling pathways.

Acknowledgments—We thank Dr. Hsing-Jien Kung (University of California-Davis) for providing the pretransformed HeLa library for two-hybrid screening. We are especially grateful to Dr. Ralf Schindler for providing the 293.CD40 cell line and the membrane preparation of CD40 ligand before publication. We thank Mik Rich for DNA sequencing. We also thank Dr. Zhaodan Cao, Merrill Ayres, Dr. Hodger Beckman, Dr. Lin Wu, and Dr. Larry Wiater for various reagents and stimulating discussion.

REFERENCES
1. Arch, R. H., Gedrich, R. W., and Thompson, C. B. (1998) Genes Dev. 12, 2821–2830
2. Wajant, H., Grell, M., and Scheurich, P. (1999) Cytokine Growth Factor Rev. 10, 15–26
3. Rhee, M., Wong, S. C., Henzel, W. J., and Goeddel, D. V. (1994) Cell 78, 681–692
4. Hu, H. M., O’Rourke, K., Boguski, M. S., and Dixit, V. M. (1994) J. Biol. Chem. 269, 30689–30672
5. Cheng, C., Cleary, A. M., Ye, Z. S., Hong, D. I., Lederman, S., and Baltimore, D. (1995) Science 267, 1494–1498
6. Mosialos, G., Birkenbach, M., Yalamanchili, R., VanArshdale, T., Ware, C., and Kieff, E. (1995) Cell 80, 389–399
7. Sato, T., Irie, S., and Reed, J. C. (1995) FEBS Lett. 358, 113–118
8. Regnier, C. H., Tomasetto, C., Moog-Lutz, C., Chenard, M. P., Wendling, C., Bassot, P., and Rin, M. C. (1995–1996) J. Biol. Chem. 270, 25715–25721
9. Ishida, T. K., Tojo, T., Aoki, T., Kobayashi, N., Ohishi, T., Watanabe, T., Yamamoto, T., and Inoue, J. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 9437–9442
10. Nakano, H., Oshima, H., Chun, W., Williams-Abbott, L., Ware, C. F., Yagita, H., and Okumura, K. (1996) J. Biol. Chem. 271, 14661–14664
11. Ishida, T., Mizushima, S., Azuma, S., Kobayashi, N., Tojo, T., Suzuki, K., Aizawa, S., Watanabe, T., Mosialos, G., Kieff, E., Yamamoto, T., and Inoue, J. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 9437–9442
12. Nakano, H., Oshima, H., Chun, W., Williams-Abbott, L., Ware, C. F., Yagita, H., and Okumura, K. (1996) J. Biol. Chem. 271, 14661–14664
13. Ishida, T., Mizushima, S., Azuma, S., Kobayashi, N., Tojo, T., Suzuki, K., Aizawa, S., Watanabe, T., Mosialos, G., Kieff, E., Yamamoto, T., and Inoue, J. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 9437–9442

FIG. 10. CD40 ligand induces dissociation of TRAF3 from the TRAF3-MIP-T3 complex. A, 293.CD40 cells were stimulated with a membrane preparation of CD40 ligand for various times as indicated. The cells were lysed, and extracts were immunoprecipitated (IP) with an anti-MIP-T3 rabbit polyclonal antibody. Coprecipitated endogenous TRAF3 was detected by immunoblotting (IB) with anti-TRAF3 antibody (top panel). The amounts of MIP-T3 proteins in the immunoprecipitates were detected by anti-MIP-T3 antibody (bottom panel). The positions of TRAF3 and MIP-T3 are indicated. B, 293.CD40 cells were stimulated with a membrane preparation of CD40 ligand for various times as indicated. The cells were lysed and extracts were immunoprecipitated with an anti-Flag antibody that recognizes the Flag-tagged CD40. Co-precipitated endogenous TRAF3 was detected by immunoblotting with anti-TRAF3 antibody (top panel). The amounts of CD40 proteins in the immunoprecipitates were detected by anti-CD40 antibody (bottom panel). The positions of TRAF3 and CD40 are indicated.
MIP-T3 Links TRAF3 to the Microtubule Network

J. (1996) J. Biol. Chem. 271, 28745–28748
12. Cao, Z., Xiong, J., Takeuchi, M., Kurama, T., and Goeddel, D. V. (1996) Nature 383, 443–446
13. Takeuchi, M., Shu, H. B., Pan, M. G., and Goeddel, D. V. (1996) J. Biol. Chem. 271, 19935–19942
14. Hsu, H., Shu, H. B., Pan, M. G., and Goeddel, D. V. (1996) Cell 84, 299–308
15. Grammer, A. C., Swantek, J. L., McFarland, R. D., Miura, Y., Geppert, T., and Lipsky, P. E. (1998) J. Immunol. 161, 1183–1193
16. Song, H. Y., Regnier, C. H., Kirschning, C. J., Goeddel, D. V., and Rothe, M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 9732–9736
17. Liu, Z. G., Hsu, H., Goeddel, D. V., and Karin, M. (1996) Cell 87, 565–576
18. Yeh, W. C., Shahinian, A., Speiser, D., Kraunus, J., Billia, F., Wakeham, A., de la Pompa, J. L., Ferrick, D., Hum, B., Losche, N., Ohashi, P., Rothe, M., Goeddel, D. V., and Mak, T. W. (1997) Immunity 7, 715–725
19. Nguyen, L. T., Duncan, G. S., Mirtsos, C., Ng, M., Speiser, D. E., Shahinian, A., Marino, M. W., Mak, T. W., Ohashi, P. S., and Yeh, W. C. (1999) Immunity 11, 379–389
20. Lee, S. Y., Reichlin, A., Santana, A., Sekol, K. A., Nussenzweig, M. C., and Choi, Y. (1997) Immunity 7, 703–713
21. Xu, Y., Cheng, G., and Baltimore, D. (1996) Immunity 5, 407–415
22. Nakano, H., Sakan, S., Kaseki, H., Takemori, T., Tada, K., Matsumoto, M., Munecihka, E., Sakai, T., Shirasawa, T., Akiba, H., Kobata, T., Santee, S. M., Ware, C. F., Rennert, P. D., Taniguchi, M., Yagita, H., and Okumura, K. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 8903–8908
23. Lomaga, M. A., Yeh, W. C., Sarosi, I., Duncan, G. S., Furlonger, C., Ho, A., Morony, S., Capparelli, C., Van, G., Kaufman, S., van der Heiden, A., Hie, A., Wakeham, A., Khon, W., Sasaki, T., Cao, Z., Penninger, J. M., Paige, C. J., Lacey, D. L., Dunstan, C. R., Boyle, W. J., Goeddel, D. V., and Mak, T. W. (1999) Genes Dev. 13, 1015–1024
24. Devigne, O., Hatzipassikou, E., Izumi, K. M., Kaye, K. M., Kleijn, M. F., Kieff, E., and Mosialos, G. (1996) Mol. Cell. Biol. 16, 7098–7108
25. Aizawa, S., Nakano, H., Ishida, T., Horie, R., Nagai, M., Ito, K., Yagita, H., Okumura, K., Inoue, J., and Watanabe, T. (1997) J. Biol. Chem. 272, 2042–2049
26. Akiba, H., Nakano, H., Nishinaka, S., Shindo, M., Kobata, T., Ateuta, M., Morimoto, C., Ware, C. F., Malinin, N. L., Wallach, D., Yagita, H., and Okumura, K. (1999) J. Biol. Chem. 274, 13353–13358
27. Arch, R. H., and Thompson, C. B. (1998) Mol. Cell. Biol. 18, 558–565
28. Bouche, L. M., Marriege, L. E., Lu, Y., Thukral, M., and Mak, T. W. (1997) Biochem. Biophys. Res. Commun. 235, 592–600
29. Mareters, S. A., Ayres, T. M., Skubatch, M., Gray, C. L., Rothe, M., and Ashkenazi, A. (1997) J. Biol. Chem. 272, 14029–14032
30. Brodeur, S. R., Cheng, G., Baltimore, D., and Thorley-Lawson, D. A. (1997) J. Biol. Chem. 272, 19777–19784
31. Darnay, B. G., Haridas, V., Ni, J., Moore, P. A., and Aggarwal, B. B. (1998) J. Biol. Chem. 273, 20551–20555
32. Wong, B. R., Josien, R., Lee, S. Y., Voigogodskaiia, M., Steinman, R. M., and Choi, Y. (1998) J. Biol. Chem. 273, 28355–28359
33. Kuhne, M. R., Robbins, M., Hambor, J. E., Mackey, M. F., Kosaka, Y., Nishiimura, T., Gigley, J. P., Noelle, R. J., and Calderhead, D. M. (1997) J. Exp. Med. 186, 337–342
34. VanArsdale, T. L., VanArsdale, S. L., Force, W. R., Walter, B. N., Mosialos, G., Kieff, E., Reed, J. C., and Ware, C. F. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 2460–2465
35. Warren, W. D., and Berton, M. T. (1995) J. Immunol. 155, 5637–5646
36. Gurus, J., Golemis, E., Chertkov, H., and Brent, R. (1993) Cell 75, 791–803
37. Rothe, M., Xiong, J., Shu, H. B., Williamson, K., Goddard, A., and Goeddel, D. V. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 8241–8246
38. Cheng, G., and Baltimore, D. (1996) Genes Dev. 10, 963–973
39. Krajewski, S., Zapata, J. M., Krajewska, M., VanArsdale, T., Shabaik, A., Gascoyne, R. D., and Reed, J. C. (1997) J. Immunol. 159, 5841–5852
40. Solomon, F., Magendantz, M., and Salzman, A. (1979) Cell 18, 431–438
41. Lewis, S. A., Ivanov, I. E., Lee, G. H., and Cowan, N. J. (1989) Nature 342, 498–505
42. Pierre, P., Pepperkok, R., and Kreis, T. E. (1984) J. Cell Sci. 107, 1909–1920
43. Ikebe, T., and Kishimoto, T. (1992) Nature 355, 733–735
44. Goode, B. L., and Feinstein, S. C. (1994) J. Cell Biol. 124, 769–782
45. Force, W. R., Cheung, T. C., and Ware, C. F. (1997) J. Biol. Chem. 272, 30835–30840
46. Shea, T. B., and Ekinci, H. F. (1998) Adv. Exp. Med. Biol. 446, 181–201
47. Cossimser, L. (1999) Curr. Opin. Cell Biol. 11, 134–141
48. Dreeses, S., Elnhet, A., and Mandelkow, E. M. (1998) Trends Biochem. Sci. 23, 307–311
49. Noble, M., Lewis, S. A., and Cowan, N. J. (1989) J. Cell Biol. 109, 3367–3376
50. Dong, C., Li, Z., Alvarez, R., Jr., Feng, X. H., and Goldschmidt-Clermont, P. J. Mol. Cell 5, 27–34
51. Kelly, R. B. (1990) Cell 61, 5–7
52. Heald, R., and Waleczak, C. E. (1999) Curr. Opin. Struct. Biol. 9, 268–274
53. Gundersen, G. G., and Thorley-Lawson, D. A. (1999) Curr. Opin. Cell Biol. 11, 81–94
54. Schoenwaelder, S. M., and Burridge, K. (1999) Curr. Opin. Cell Biol. 11, 274–286