RIP2 Is a Novel NF-κB-activating and Cell Death-inducing Kinase*

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Through specific interactions with members of the tumor necrosis receptor (TNFR) family, adapter molecules such as the serine/threonine (Ser/Thr) kinase RIP mediate divergent signaling pathways including NF-κB activation and cell death. In this study, we have identified and characterized a novel 61-kDa protein kinase related to RIP that is a component of both the TNFR-1 and the CD40 signaling complexes. Receptor interacting protein-2 (RIP2) contains an N-terminal domain with homology to Ser/Thr kinases and a C-terminal caspase activation and recruitment domain (CARD), a homophilic interaction motif that mediates the recruitment of caspase death proteases. Overexpression of RIP2 signaled both NF-κB activation and cell death. Mutational analysis revealed the pro-apoptotic function of RIP2 to be restricted to its C-terminal CARD domain, whereas the intact molecule was necessary for NF-κB activation. RIP2 interacted with other members of the TNFR-1 signaling complex, including inhibitor of apoptosis protein cIAP1 and with members of the TNFR-associated factor (TRAF) family, specifically TRAF1, TRAF5, and TRAF6, but not with TRAF2, TRAF3, or TRAF4. These TRAF interactions mediate the recruitment of RIP2 to receptor signaling complexes.

Members of the tumor necrosis factor receptor (TNFR) family include TNFR-1 and -2 (1), Fas (CD-95/APO-1) (2, 3), lymphotoxin-β receptor (4), CD40 (5, 6), CD30 (7), OX-40 (8), DR3 (9), DR4 (10), and DR5 (11, 12), and play an important role in overlapping cellular responses, including cell activation, proliferation, differentiation, NF-κB activation, and apoptosis. TNFR family members are defined by the presence of cysteine-rich repeats in their extracellular domain. Certain members share additional homology, possessing an intracellular domain termed the “death domain” that mediates recruitment of death domain-containing adapter molecules to the receptor signaling complex (13–15). TNFR-1 can signal a diversity of cellular activities by assembling an intricate signaling complex made of a number of adapter molecules that enables mediation of both apoptosis and NF-κB activation. CD40 is a cell surface transmembrane glycoprotein receptor expressed in late B-cells in the bone marrow, mature B-cells and certain accessory cells including bone marrow-derived dendritic cells and follicular dendritic cells (16–18). CD40 activation is necessary for B-cell proliferation and immunoglobulin class switching (5, 6, 19).

The absence of an enzymatic domain in the cytoplasmic region of the TNFR family implies that signaling is mediated by receptor-associating proteins. The intracellular mediators identified to date fall into two distinct groups. The first group consists of proteins with a highly conserved domain termed the death domain and includes TRADD (20), FADD/MORT1 (13), RIP (21, 22), and RAIDD (23). These molecules are recruited to TNFR-1 or to Fas (CD-95) through homophilic interactions involving the cognate death domains. Overexpression of these death domain-containing adapter molecules mimics responses induced by ligand-receptor interactions, including NF-κB activation and apoptosis (22–24). In contrast to the other adapter molecules, RIP, in addition to a death domain, contains an N-terminal region of approximately 300 residues that is homologous to Ser/Thr protein kinases. RIP possesses kinase activity as it autophosphorylates itself on Ser/Thr residues. Overexpression of RIP engages the death pathway and activates NF-κB. The kinase domain does not appear to mediate either function, inasmuch as overexpression of the death domain by itself is sufficient to induce apoptosis and the intermediate domain (which separates the kinase and death domains) mediates NF-κB activation.

The second group consists of the TNF-receptor-associated factors (TRAF); to date, six members have been identified. TRAF1 and TRAF2 were initially identified based on their interaction with the cytoplasmic domain of TNFR-2 (25). TRAF1 and TRAF2 form homodimeric and heteromeric complexes with each other. Importantly, TRAF2, but not TRAF1, binds directly to the cytoplasmic domain of TNFR-2 and CD40 (26). Therefore, TRAF1 can only be recruited to the receptor signaling complex through TRAF2. TRAF3 interacts with CD40, CD30, TNFR-2, and lymphotoxin-β receptor (27, 28), but its signaling function remains unclear. TRAF5 mediates signals arising from CD40 and lymphotoxin-β receptor (29, 30), whereas TRAF6 is involved in both CD40 and interleukin 1 receptor signaling (31, 32). TRAF2, TRAF5, and TRAF6 mediate NF-κB activation (26, 29, 31, 32).

TRAF family members in turn associate with downstream signaling components including the cellular inhibitors of apoptosis, cIAP1 and cIAP2, present in both TNFR-1 and TNFR-2 signaling complexes (33). The IAPs, being caspase inhibitors, could potentially act to attenuate apoptosis. Herein, we describe the identification of a novel TRAF-interacting kinase, designated RIP2, that specifically binds to TRAF1, TRAF5, and TRAF6, and is recruited to the TNFR-1 and CD40 receptor signaling complexes.

**MATERIALS AND METHODS**

Cloning of Human RIP2—The cDNA corresponding to a partial open reading frame of the C terminus of RIP2 was identified as a sequence

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∥ The abbreviations used are: TNFR, tumor necrosis factor receptor; PCR, polymerase chain reaction; HUVEC, human umbilical ven endothelial cell; HA, hemagglutinin; mAb, monoclonal antibody; IAP, inhibitor of apoptosis; cIAP, cellular inhibitor of apoptosis; TRAF, TNFR-associated factor; CARD, caspase activation and recruitment domain.
homologous to RIP (21, 22) on searching the Human Genome Sciences data base using established expressed sequence tag methods (34). A full-length cDNA was obtained by screening an oligo(dT)-primed human umbilical vein endothelial cell (HUVEC) cDNA library. A total of 1.310⁶ transformants were screened with a 32P-labeled DNA fragment generated by PCR corresponding to amino acids 82–248 of the RIP2 open reading frame (35). Double-stranded DNA sequencing was carried out by the dideoxy chain termination method using modified T7 DNA polymerase (Sequenase, U. S. Biochemical Corp.). Sequence alignments were performed using DNASTAR Megalign software.

**Northern Blot Analysis**—Human multiple tissue and human cancer cell line poly(A)⁺ RNA blots (CLONTECH) containing 2.5 mg/lane poly(A)⁺ RNA were hybridized according to the manufacturer’s instructions using a 32P-labeled RIP2 probe corresponding to amino acids 82–248 of the RIP2 open reading frame (35).

**Expression Vectors**—The DNA inserts encoding the N-terminal HA-tagged (HA-RIP2) or C-terminal Myc-His₆-tagged (Myc-RIP2) RIP2 eukaryotic expression constructs were generated by standard PCR techniques and subcloned into the mammalian expression vectors pcDNA3 or pcDNA3.1/Myc-His (Invitrogen), respectively. Alteration of the catalytic lysine 47 to an alanine for RIP2 (K47A) was accomplished by site-directed mutagenesis employing a four-primer PCR-based method (36). The mutagenetic oligonucleotides were GTCCAGGTGGCCGTGGCCCACCTGCACATCCACA and TGTGGATGTGCAGGTGGGCCACG GCCACCTGGAC. The presence of the introduced mutation (underlined) and fidelity of PCR replication was confirmed by sequence analysis.

**Cell Death Assays**—Human MCF7 breast carcinoma cells were transiently transfected as described previously (13, 37). Briefly, 2.5 × 10⁵ MCF7 cells were transfected with 0.1 μg of the reporter plasmid pcMV β-galactosidase plus 1 μg of test plasmid in six-well tissue culture dishes using LipofectAMINE as per manufacturer’s instructions. Thirty-six hours after transfection, the cells were fixed with 0.5% gluteraldehyde and stained with 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside for 3–4 h. Cells were visualized by phase contrast microscopy. Approximately 300 β-galactosidase-positive cells were assessed from each transfection (n = 3) from three randomly selected fields. The presence of the introduced mutation (underlined) and fidelity of PCR replication was confirmed by sequence analysis.
fields, and the mean of these was used to calculate percentage apoptosis. Viable or apoptotic cells were distinguished based on morphological alterations typical of adherent cells undergoing apoptosis including becoming rounded, condensed, and detached from the dish (38, 39).

Co-immunoprecipitation and Western Blot Analysis—Transient transfection of 293 cells was performed by calcium phosphate precipitation with the indicated constructs as described previously (40). Where indicated, a CrmA expression construct was included to suppress apoptosis. Cells were harvested 24–36 h after transfection and lysed in 1 ml of lysis buffer (50 mM HEPES, 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, and a protease inhibitor mixture). Lysates were immunoprecipitated with control monoclonal antibody (mAb) (designated C) or epitope mAb for 4 h at 4 °C as described (24). The precipitates were washed three times in lysis buffer and resolved by SDS-polyacrylamide gel electrophoresis. Subsequent protein immunoblotting was performed as described (24).

NF-κB Luciferase Assay—For reporter gene assays, 293 cells (2.5 × 10^6 cells/well) were seeded into six-well plates and transfected with control pcDNA3 vector (lane 1) or expression vectors encoding the RIP2 (K47A) point mutant (lane 2) or HA epitope-tagged wild type RIP2 (lane 3). Cell lysates were immunoprecipitated 36 h after transfection with anti-HA monoclonal antibodies. The immunoprecipitates were subject to in vitro kinase assays (top) or Western blot analysis with anti-HA polyclonal antibodies. B, alignment of RIP2 to the catalytic domains of RIP, Ser/Thr, and Tyr kinases. Consensus residues conserved in over 95% of sequences analyzed are indicated in bold.

NF-κB Luciferase Assay—For reporter gene assays, 293 cells (2.5 × 10^6 cells/well) were seeded into six-well plates and transfected by the calcium phosphate precipitation method (40) with 0.1 μg of E-selectin-luciferase reporter gene plasmid and the indicated amounts of each expression construct. The total DNA concentration was kept constant by supplementation with empty vector. Cells were harvested 24 h after transfection and reporter gene activity determined with the Luciferase Assay System (Promega). A β-galactosidase expression vector (0.1 μg) was used to normalize transfection efficiencies.

In Vitro Kinase Assay—Immunoprecipitates were prepared from transfected 293 cells as described above and washed once with 1 ml of kinase assay buffer (50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 6 mM MgCl₂, 1 mM MnCl₂, and 1 mM dithiothreitol). The kinase assay was performed at 30 °C for 30 min in 30 μl of kinase assay buffer supplemented with 100 mM [γ-32P]ATP and 5 μM ATP. Reactions were stopped with 20 μl of SDS sample buffer, boiled for 5 min, and analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography.
RESULTS AND DISCUSSION

Cloning and Structure of Human RIP2—Data base searching revealed two novel cDNA clones (HDEDU78 and HNFJ62) encoding partial open reading frames that had homology to the serine threonine kinase domain of RIP. A HUVEC cDNA library was screened to obtain a full-length cDNA. It contained a 1623-base pair open reading frame encoding a novel 541-residue protein with a predicted molecular mass of 61 kDa and was designated RIP2 (Fig. 1A). The putative initiator methionine (ACCATGGA) was in agreement with the consensus Kozak sequence for translation initiation (41). The N terminus of RIP2 displayed high homology to protein kinases, with residues +1 to +311 of RIP2 constituting a protein kinase domain. Alignment of the predicted kinase domain of RIP2 with that of both human and murine RIP (Fig. 1B) demonstrated that the three molecules share significant homology and conservation. The middle of the molecule contains a stretch of 143 amino acids with little or no homology to known proteins.

The C Terminus of RIP2 Contains a CARD Motif—A BLAST search of the public data base revealed that the 87 C-terminal amino acids of RIP2 had statistically significant homology (p < 0.001) with the intermediate domain of both mammalian cIAP1 and cIAP2 (Fig. 1C) and, to a lesser extent, similarity to the prodomain of the human death protease caspase-2 and the Caenorhabditis elegans death protease CED-3. Comparable domains of approximately 90 amino acids have been identified in a number of other molecules involved in apoptotic signaling, including RAIDD, caspase-1, caspase-2, caspase-9, and cIAP1 and cIAP2. This domain is known to mediate homophilic interactions, allowing for the recruitment of caspases to receptor complexes. Therefore, this unique motif has been termed CARD (for caspase activation and recruitment domain) (42).

Sequence comparisons revealed that the RIP2 CARD domain possessed highest homology with the corresponding domain in cIAP1 and cIAP2 (51.3% and 47.9% similarity). This was comparable to the homology between the CARD motifs that mediate the interaction of RAIDD and caspase-2 (67.2% similarity).

Tissue Distribution of RIP2—Human tissue and cell line RNA blots were probed with a 32P-labeled cDNA probe specific complexes. Therefore, this unique motif has been termed actions, allowing for the recruitment of caspases to receptor including RAIDD, caspase-1, caspase-2, caspase-9, and cIAP1

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Tissue Distribution of RIP2—Human tissue and cell line RNA blots were probed with a 32P-labeled cDNA probe specific for RIP2. RIP2 was found to be constitutively expressed in a variety of human tissues (Fig. 2). Two transcripts were detected. The 2-kilobase transcript corresponds in size to the cDNA cloned from the HUVEC library. The other transcript, which is approximately 2.4 kilobases, may represent a related RIP2 isoform or may have arisen from use of an alternate polyadenylation signal. Nevertheless, RIP2 was highly expressed in the spleen, peripheral blood leukocytes, placenta, testis, and heart, but was barely detectable in the thymus (Fig. 2, upper panels). A variety of transformed cell lines expressed low levels of RIP2, whereas RAJ1 cells, a transformed B-cell line, displayed significant expression (Fig. 2, lower panel).

RIP2 Is a Protein Kinase—293 cells were transfected with HA-RIP2 or HA-RIP2(K47A), a mutant in which the conserved lysine essential for enzymatic activity and ATP binding has been altered to an alanine, and immunoprecipitated. An in vitro kinase assay was performed on the immunoprecipitated complex, and a 61-kDa 32P-labeled band corresponding to RIP2 was identified (Fig. 3A). As predicted, no kinase activity was observed for RIP2(K47A). These observations demonstrate that RIP2 is an autophosphorylating protein kinase. Based on sequence alignments to the catalytic domains of known protein kinases (Fig. 3B), RIP2 contains residues that are highly conserved in Ser/Thr kinases. Specifically, the key subdomains that differentiate Tyr from Ser/Thr substrate specificity (the DLKTQN sequence, corresponding to kinase subdomain VI, and the GTIYMPPE sequence, corresponding to kinase subdomain VIII) are conserved (Fig. 3B) (43, 44).

RIP2-induced Apoptosis Is Mediated through Its CARD Motif—To study its functional role, human MCF7 breast carcinoma cells were transiently transfected with the reporter gene β-galactosidase and either epitope-tagged RIP2 alone or empty vector. Transfected cells were stained with 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside and examined by phase contrast microscopy. B, MCF7 cells were transiently transfected with the indicated construct and the β-galactosidase reporter construct used as a marker for transfection. Cells were fixed and the morphology of 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside-stained cells examined by light microscopy. Data (mean ± S.E.) shown are the percentage of apoptotic cells among the total number of cells counted (n = 3). C, MCF7 cells were transiently transfected with RIP2 or RIP epitope-tagged constructs and a 4-fold molar excess of the indicated apoptosis inhibitors. The broad spectrum interleukin 1-convertase enzyme family inhibitor z-VAD-fmk (20 μM) was added to the cell cultures 5 h after transfection.
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Fig. 5. Overexpression of RIP2 activates NF-κB. A, induction of NF-κB reporter activity by RIP2. 293 cells were transiently cotransfected with a pELAM-luciferase reporter gene, β-galactosidase, and the indicated amounts of RIP2 expression vector. The total concentration of transfected DNA was kept constant by adding empty vector. Data represent luciferase activities, normalized for β-galactosidase expression, and are shown for a representative experiment. B, 293 cell cultures were transfected with equal amounts of RIP2 expression vector and cotransfected with the indicated amount of TRAF2-DN, TRAF3-DN, TRAF6-DN, NIK-DN, RIP-DN, or FADD-DN expression vectors. The total concentration of transfected DNA was kept constant by adding empty vector. Data represent luciferase activities, normalized for β-galactosidase expression.

C-terminal CARD motif (Fig. 4B). Kinase activity was not required as RIP2(K47A) induce apoptosis at comparable levels to wild-type RIP2 (Fig. 4B). Furthermore, deletion of the kinase domain of RIP2 (positions 311–541) had no effect on its pro-apoptotic activity. Taken together, these results substantiate the previous observation that the CARD domain is both sufficient and necessary for RIP2-mediated apoptosis. RIP2-induced apoptosis was blocked by a broad range of known inhibitors of apoptosis, including CrmA, z-VAD-fmk, p35, bcl-xl, cIAP1, and eIAP2 (Fig. 4C).

RIP2 Induces NF-κB—Because RIP2 has sequence homology to RIP, a known activator of NF-κB (22), we investigated a possible role for RIP2 in NF-κB activation. An NF-κB-dependent E-selectin-luciferase reporter construct and a RIP2 expression vector were cotransfected into 293 cells. Expression of RIP2 activated the reporter gene in a dose-dependent manner (Fig. 5A), with maximum induction of luciferase activity being 40-fold compared with vector control. The intact molecule was required for this activity, as truncated derivatives of RIP2 failed to induce NF-κB activity, suggesting that integrity of the molecule was essential for its ability to signal NF-κB (data not shown). RIP2(K47A) retained the ability to induce NF-κB activation, consistent with kinase activity not being required (data not shown).

NF-κB activation induced by members of the TNFR family is in part mediated by the TRAF adapter family. We therefore determined whether dominant negative versions of TRAF proteins could act as inhibitors of RIP2-induced NF-κB activation. RIP2-induced NF-κB activation was blocked by dominant negative derivatives of TRAF2 (TRAF2-DN) and TRAF6 (TRAF6-DN), whereas, as expected, TRAF3-DN did not interfere with RIP2-mediated NF-κB activation (Fig. 5B). Furthermore, a dominant negative mutant version of the downstream kinase NIK, which is implicated in TRAF-mediated NF-κB activation, strongly inhibited RIP2-induced NF-κB activation. Conversely, dominant negative versions of adapter molecules that act upstream of the TRAFs (RIP-DN and FADD-DN) were without effect.

RIP2 Interacts with Inhibitor of Apoptosis Protein cIAP1—Several proteins involved in apoptotic signaling possess regions of sequence similarity that mediate homophilic interactions. Frequently, these domains mediate the association of proteins in a signaling cascade. RIP2 contains a CARD motif that is known to mediate protein-protein interactions (23). Given this, we determined if RIP2 might interact with other CARD-containing molecules. 293 cells were transiently transfected with expression constructs that directed the synthesis of Flag-RAIDD, Flag-caspase-9, Flag-caspase-1, Flag-caspase-4, Myc-cIAP1, Myc-cIAP2, and epitope-tagged-RIP2. Immunoprecipitation analysis revealed surprising specificity, with RIP2 binding only cIAP1 (Fig. 6), the molecule to which it is most similar in the CARD motif.

RIP2 Interacts with TRAF Proteins—Previous studies have demonstrated that RIP interacts with TRAF1, TRAF2, and TRAF3 and, as part of a RIP-TRAF2-TRADD complex, can be recruited to TNFR-1 (22). Because of the observed sequence similarity between RIP and RIP2, we determined if RIP2 could similarly interact with TRAF proteins. 293 cells were transiently transfected with expression constructs that directed the synthesis of Flag-epitope-tagged TRAF proteins and Myc-epitope-tagged-RIP2. Immunoprecipitation of Flag-TRAF1, Flag-TRAF3, and Flag-TRAF6 quantitatively coprecipitated Myc-RIP2 (Fig. 7A). No association was detected with Flag-TRAF2, Flag-TRAF3, or Flag-TRAF4 (Fig. 7A). TRAF2, TRAF3, and TRAF6 mediate NF-κB activation (26, 29–31). Taken together, these studies raised a paradox inasmuch as RIP2-induced NF-κB activity can be inhibited by TRAF2-DN (Fig. 4B), despite the absence of a direct interaction between RIP2 and TRAF2 (Fig. 7A). However, because TRAF1 interacts strongly with both TRAF2 and RIP2, it was possible that
TRAF2 interacted with RIP2 through TRAF1. 293 cells were transfected with combinations of expression constructs that directed the synthesis of Myc-RIP2, Flag-TRAF1, and AU1-TRAF2. Cell lysates were immunoprecipitated with the indicated antibodies and the immunoprecipitates analyzed by Western blotting using anti-Myc antibody (Fig. 7B). This anal-

**Fig. 6. RIP2 interacts with inhibitor of apoptosis protein cIAP1.** Coimmunoprecipitation of RIP2 with CARD-containing proteins. 293 cells (2.5 x 10⁶) were cotransfected with Flag-RAIDD, Flag-caspase-9, Flag-caspase-1, Flag-caspase-2, Myc-cIAP1 or Myc-cIAP2, and epitope-tagged RIP2 expression constructs. Thirty-six hours after transfection, extracts were prepared and immunoprecipitated (IP) with a control mAb (designated C) or a mAb to the specified epitope tag. Expression of the indicated proteins is shown in the upper insets.

**Fig. 7. RIP2 interacts with TRAF proteins. A,** coimmunoprecipitation of RIP2 with TRAF proteins. 293 cells (2.5 x 10⁶) were cotransfected with Myc-RIP2 and the indicated Flag-TRAF expression constructs. Thirty-six hours after transfection, extracts were prepared and immunoprecipitated (IP) with a control mAb (designated C) or a mAb to the specified epitope tag. Coprecipitating Myc-RIP2 was detected by immunoblotting with anti-Myc monoclonal antibody. Expression of Flag-TRAF proteins are shown in the upper insets. B, TRAF1 recruits RIP2 to TRAF2. Through a RIP2-TRAF1-TRAF2 complex, RIP2 can be recruited to TRAF2. 293 cells (2.5 x 10⁶) were cotransfected with the indicated combination of expression plasmids for Myc-RIP2, Flag-TRAF1, and AU1-TRAF2. Thirty-six hours after transfection, extracts were prepared and immunoprecipitated (IP) with a control mAb (designated C) or a mAb to the specified epitope tag. Coprecipitating Myc-RIP2 was detected by immunoblotting with horseradish peroxidase-conjugated anti-Myc monoclonal antibody. Expression of TRAF proteins was confirmed by immunoblotting as in A.
ysis confirmed that RIP2 associated strongly with TRAF1, but not with TRAF2. However, when cells coexpressed RIP2, TRAF1, and TRAF2, communoprecipitation of RIP2 with TRAF2 was evident (Fig. 7B), suggesting that TRAF1 can serve as a bridging molecule between RIP2 and TRAF2.

**RIP2 Is Recruited to Both TNFR1- and CD40-Receptor Complexes**—RIP2 specifically binds to TRAF5 and TRAF6 and, through TRAF1, interacts with TRAF2. Therefore, we determined whether RIP2 could be communoprecipitated with members of the TNFR family in the concomitant presence of TRAF molecules. In 293 cells expressing RIP2, CD40, and TRAF6 or TRAF5, RIP2 was quantitatively communoprecipitated with CD40 (Fig. 8A). In cells expressing CD40, RIP2, and TRAF1 or TRAF2 only, a weak association (presumably mediated by endogenous TRAFs) could be detected between CD40 and RIP2. However, when CD40 and RIP2 were expressed in the presence of both TRAF1 and TRAF2, enhanced association of RIP2 with CD40 could be detected (Fig. 8B).

On activation of TNFR-1, the adapter molecule TRADD is recruited to the signaling complex where it subsequently binds the TRAF2/TRAF1 heterocomplex. Because RIP2 binds to the same heterocomplex, we asked if it could be recruited to TNFR-1. In 293 cells expressing TNFR-1, TRADD, and TRAF1 or TRAF2, little RIP2 communoprecipitated with TNFR-1 (Fig. 8C). The small amount that did precipitate was probably mediated by endogenous TRAFs. However, when TNFR-1 and RIP2 were co-expressed in the presence of TRADD, TRAF1, and TRAF2, enhanced binding of RIP2 to TNFR-1 was detected (Fig. 8C). Taken together, these results suggest that RIP2 could be a component of both the TNFR-1 and CD40 receptor signaling complexes.

In summary, we have identified a novel protein kinase, RIP2, that can be recruited to the CD40 and TNFR-1 signaling complexes. Because TRAF proteins function as adaptor molecules for other TNFR family members, it is possible that RIP2 is also recruited to additional receptors. Regardless, the identification of this molecule has added a second kinase (other than RIP) that is recruited to signaling complexes assembled by certain members of the TNFR family.
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