

All TRAFs are not created equal: common and distinct molecular mechanisms of TRAF-mediated signal transduction

Jee Y. Chung, Young Chul Park, Hong Ye and Hao Wu

Department of Biochemistry, Weill Medical College of Cornell University, New York, NY 10021, USA
Author for correspondence (e-mail: haowu@med.cornell.edu)

Journal of Cell Science 115, 679-688 (2002) © The Company of Biologists Ltd

Summary

The tumor necrosis factor (TNF) receptor associated factors (TRAFs) have emerged as the major signal transducers for the TNF receptor superfamily and the interleukin-1 receptor/Toll-like receptor (IL-1R/TLR) superfamily. TRAFs collectively play important functions in both adaptive and innate immunity. Recent functional and structural studies have revealed the individuality of each of the mammalian TRAFs and advanced our understanding of the underlying molecular mechanisms.

Here, we examine this functional divergence among TRAFs from a perspective of both upstream and downstream TRAF signal transduction pathways and of signaling-dependent regulation of TRAF trafficking. We raise additional questions and propose hypotheses regarding the molecular basis of TRAF signaling specificity.

Key words: TRAF, TNF, IL-1R/TLR, NF- κ B, AP-1

Introduction

The tumor necrosis factor (TNF) receptor associated factors (TRAFs) constitute a family of genetically conserved adapter proteins that has been found in mammals (TRAF1-6, see review (Arch et al., 1998)), as well as in other multicellular organisms such as *Drosophila* (Liu et al., 1999; Grech et al., 2000; Medzhitov and Janeway, 2000; Zapata et al., 2000), *Caenorhabditis elegans* (Wajant et al., 1998) and *Dictyostelium discoideum* (Regnier et al., 1995). Mammalian TRAFs have emerged as the major signal transducers for the TNF receptor superfamily and the interleukin-1 receptor/Toll-like receptor (IL-1R/TLR) superfamily (Table 1). A wide range of biological functions, such as adaptive and innate immunity, embryonic development, stress response and bone metabolism, are mediated by TRAFs through the induction of cell survival, proliferation, differentiation and death. TRAFs are also involved in the signal transduction of the Epstein-Barr virus transforming protein LMP-1 (Mosialos et al., 1995). In *Drosophila*, TRAFs are essential for dorsoventral polarization and innate host defense by the signal transduction initiated through the Toll receptor (Imler and Hoffmann, 2001; Preiss et al., 2001).

The TRAF proteins are characterized by the presence of a novel TRAF domain at the C-terminus, which consists of a coiled-coil domain followed by a conserved TRAF-C domain (Rothe et al., 1994) (Fig. 1). The TRAF domain plays an important role in TRAF function by mediating self-association and upstream interactions with receptors and other signaling proteins (Takeuchi et al., 1996). The N-terminal portion of most TRAF proteins contains a RING finger and several zinc finger motifs, which are important for downstream signaling events (Rothe et al., 1995; Takeuchi et al., 1996).

Many of the biological effects of TRAF signaling appear to be mediated through the activation of transcription factors of

the NF- κ B and AP-1 family. NF- κ B promotes the expression of genes involved in inflammatory and anti-apoptotic responses (Baeuerle and Baltimore, 1996; Beg and Baltimore, 1996; Liu et al., 1996). It is activated by the I κ B kinase (IKK), which consists of two kinase subunits, IKK α and IKK β , and a regulatory subunit, IKK γ /NEMO (DiDonato et al., 1997; Regnier et al., 1997; Zandi et al., 1997; Krappmann et al., 2000). Phosphorylation and degradation of I κ B lead to the release and translocation of NF- κ B to the nucleus to activate transcription (Stancovski and Baltimore, 1997). AP-1 activity is stimulated by mitogen-activated protein (MAP) kinases through either direct phosphorylation or transcription of AP-1 components (Karin, 1996). MAP kinases, which include Ser/Thr kinases such as JNKs/SAPKs, ERKs and p38s, are at the downstream end of a three-tiered system that also contains MAP kinase kinase (MAP2K) and MAP kinase kinase kinase (MAP3K). The stimulation of AP-1 activity by MAP kinases may elicit stress responses and promote both cell survival and cell death (Shaulian and Karin, 2001).

As adapter proteins, TRAFs elaborate receptor signal transduction by serving as both a convergent and a divergent platform. Therefore, different TRAFs are created with their own specific biological roles. Their distinct upstream and downstream signaling pathways may determine this specificity. Recent structural and biochemical data have provided us with a much better understanding of the upstream signaling mechanism of TRAFs. Many of the current studies of TRAF downstream signaling focus on the activation of NF- κ B and AP-1 transcription factors. However, accumulating evidence points to the differential regulation of this apparently common downstream pathway as well as to additional TRAF-specific pathways for eliciting different biological functions. We further suggest that signaling-dependent TRAF trafficking may be another crucial regulatory factor. This commentary will focus

Table 1. Current members of the TNF receptor and IL-1R/TLR superfamilies

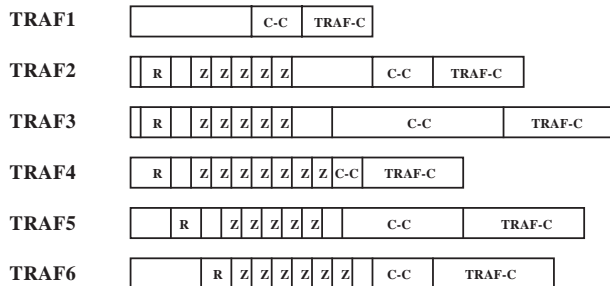
TNF receptor superfamily	
Receptors with intracellular death domains: TNFR1, Fas, DR3, DR4, DR5, DR6, NGFR	
Receptors with no intracellular death domains: TNFR2, LT β R, CD40, CD30, OX40, CD27, 4-1BB, RANK/TRANCE-R, Troy, HveA, EDAR, XEDAR, AITR, TACI, BCMA	
IL-1R/TLR superfamily	
IL-1 receptor family: IL-1R, IL-1RAcP, IL-18R, IL-18RAcP	
Toll-like receptor family: TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10	

on the common and distinct molecular mechanisms of TRAF-mediated signal transduction. For complementary information, please refer to other recent reviews on TRAFs and TNF receptors (Wallach et al., 1999; Inoue et al., 2000; Locksley et al., 2001; Wajant et al., 2001).

Specific biological functions of mammalian TRAFs

Mammalian TRAF1 and TRAF2 were originally identified by their association with TNFR2 (Rothe et al., 1994). The other mammalian TRAFs were identified as follows: TRAF3 by its interaction with CD40 and the Epstein-Barr virus transforming protein LMP1 (Cheng et al., 1995; Mosialos et al., 1995; Sato et al., 1995); TRAF4 by its overexpression in breast carcinoma cells (Regnier et al., 1995); TRAF5 by its interaction with CD40 and LT β R (Ishida et al., 1996; Nakano et al., 1996; Mizushima et al., 1998) and TRAF6 by its participation in the signal transduction of CD40 and interleukin-1, a cytokine that is not related to TNF (Cao et al., 1996b; Ishida et al., 1996).

A.



B.

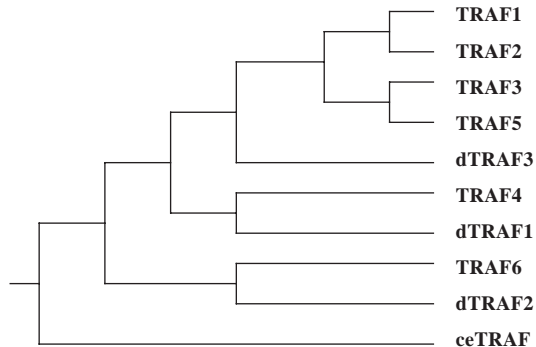


Fig. 1. Sequence characteristics of TRAFs. (A) Domain organization of the mammalian TRAFs. R, RING domain; Z, zinc-finger domain; C-C, coiled-coil domain; TRAF-C, TRAF-C domain. The TRAF domain comprises the coiled-coil domain and the TRAF-C domain. (B) The proposed evolutionary tree for the mammalian, *Drosophila* and *C. elegans* TRAFs. This figure is adapted from Grech et al. (Grech et al., 2000).

However, further extensive studies have shown that the specific biological function of each TRAF protein is not necessarily related to its origin of identification (Table 2, Fig. 2).

Since its discovery, TRAF2 has become the prototypical member of the TRAF family. The paradigm of TRAF-mediated NF- κ B and MAP kinase activation was first demonstrated using both TRAF2 overexpression and a dominant-negative phenotype of a TRAF2 derivative lacking the RING domain (Rothe et al., 1995; Hsu et al., 1996b; Takeuchi et al., 1996; Duckett et al., 1997; Reinhard et al., 1997; Arch et al., 1998). TRAF2 transcripts have been detected in almost every tissue (Rothe et al., 1994), making TRAF2 the most widely expressed TRAF family member.

TRAF2 plays a cytoprotective role, which was demonstrated by the premature death of TRAF2-deficient mice owing to severe runting. In addition, TRAF2-deficient cells are highly sensitive to TNF-induced cell death (Yeh et al., 1997). The lack of TRAF2 or the expression of a dominant-negative form of TRAF2 only led to a modest defect in TNF-induced NF- κ B activation but resulted in a severe reduction of JNK/SAPK activation (Lee et al., 1997; Yeh et al., 1997; Devin et al., 2000). Recent data suggest that TRAF2 is important for NF- κ B activation, but this role may be partially compensated for by the highly related TRAF5 (see below) (Nakano et al., 2000). The sensitization to TNF-induced cell death in the absence of TRAF2 must have been largely due to an NF- κ B-independent mechanism (Lee et al., 1997; Yeh et al., 1997; Lee et al., 1998). One possibility may be related to the failure to recruit other proteins such as cellular inhibitors of apoptosis proteins (cIAPs) to the TNFR1 receptor signaling complex in the absence of TRAF2 (Wang et al., 1998; Park et al., 2000). TNF toxicity through TNFR1 appears to contribute significantly to the survival defects in TRAF2-deficient mice because a double

Table 2. Summary of TRAF functions

TRAFs	Implicated functions
TRAF1	Apoptotic protection Feedback regulation of receptor signaling
TRAF2	Anti-apoptotic signaling JNK activation Perinatal survival
TRAF3	T-cell-dependent antigen response Perinatal survival
TRAF4	Tracheal formation
TRAF5	CD27 and CD40 signaling
TRAF2 and 5	NF- κ B activation
TRAF6	Bone metabolism CD40 signaling IL-1 signaling LPS signaling Perinatal survival

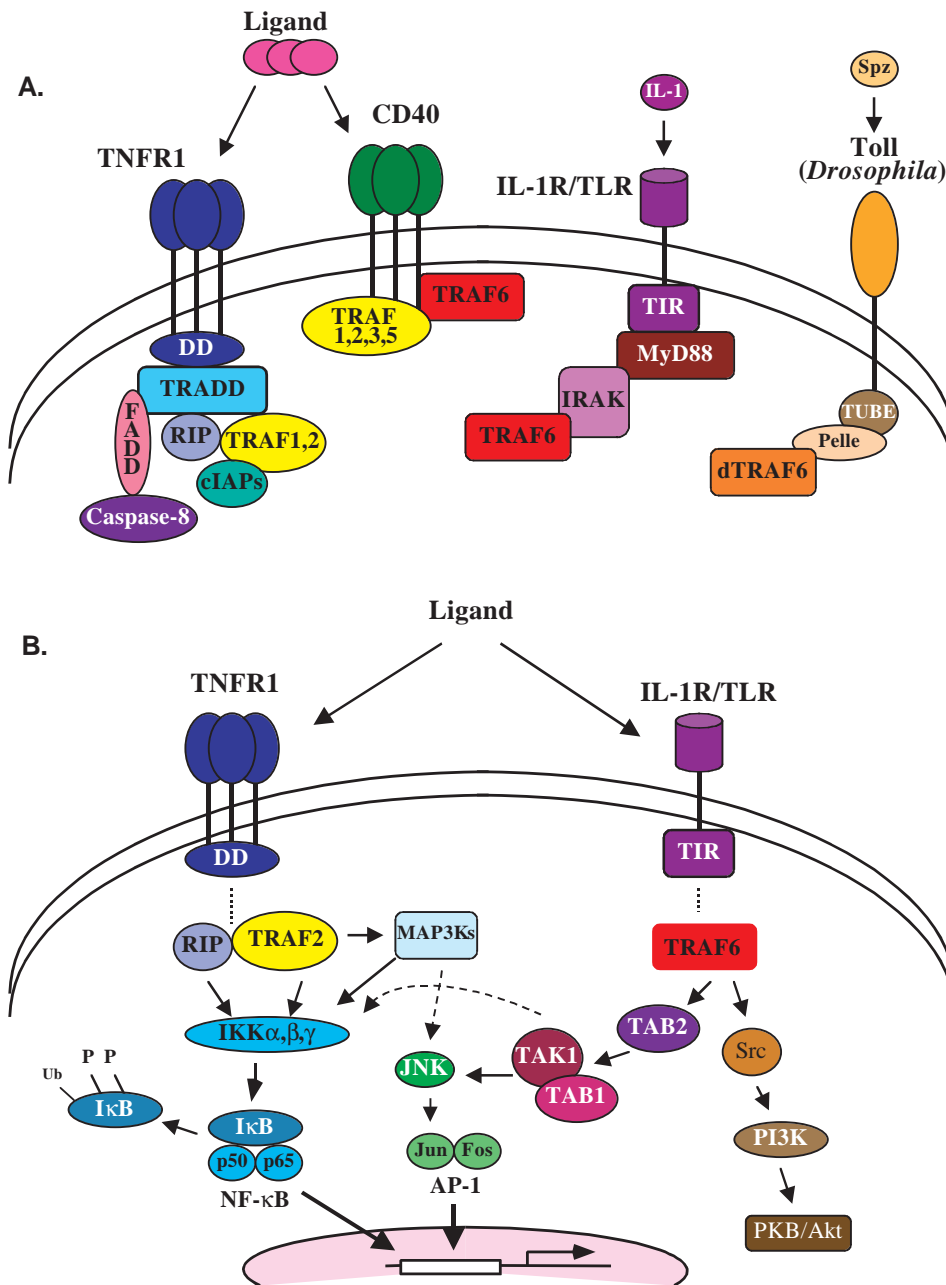


Fig. 2. TRAF signaling pathways. (A) Membrane-proximal events in TRAF signaling, showing direct receptor-TRAF recruitment and indirect receptor-TRAF interactions. (B) Downstream signaling events for TRAFs, shown here for two representative TRAF family members, TRAF2 and TRAF6.

are specifically recruited through TRAF1 and TRAF2 (Wang et al., 1998; Park et al., 2000).

Although TRAF3 possesses a putative domain organization similar to TRAF2 and TRAF5, overexpression of TRAF3 did not activate NF- κ B (Rothe et al., 1995). In contrast, it was reported that TRAF3 recruitment to LT β R led to cell death (Force et al., 1997), and that both N- and C-terminal domains of TRAF3 negatively regulate NF- κ B activation induced by Ox40 (Takaori-Kondo et al., 2000). However, it has also been shown that there are a variety of mRNA species of TRAF3 and that some splice variants do induce NF- κ B activation (van Eyndhoven et al., 1999). Similar to TRAF2-deficient mice, TRAF3-deficient mice have poor perinatal and neonatal survival (Xu et al., 1996). However, despite the runting phenotype and the hypotrophy of the spleen and thymus, which is similar to the phenotype displayed by TRAF2-deficient mice, the immune system is fairly normal except in the T-cell-dependent antigen responses (Xu et al., 1996).

The biological importance of TRAF4 was revealed by the gross tracheal malformation displayed by TRAF4-deficient mice (Shiels et al., 2000), which suggested a parallel

function of TRAF4 with the *Drosophila* Toll pathway in body organization. Analysis of TRAF4 expression has also implicated TRAF4 in the function of neural multipotent cells and epithelial stem cells in adult mammals (Krajewska et al., 1998; Masson et al., 1998). Even though there is evidence that TRAF4 may interact with several receptors in the TNF receptor superfamily (Krajewska et al., 1998; Ye et al., 1999), further studies are required to elucidate the molecular pathway of TRAF4 signaling.

TRAF5 is considered to be a close functional and structural homologue of TRAF2, and overexpression of TRAF5 can also activate NF- κ B and AP-1 transcription factors (Ishida et al., 1996; Nakano et al., 1996). However, deletion of TRAF5 did not cause perinatal lethality, perhaps owing to the more restricted expression pattern of TRAF5 compared with TRAF2 (Ishida et al., 1996; Nakano et al., 1996). TRAF5 deficiency

deficiency in TRAF2 and TNFR1 resulted in increased survival (Yeh et al., 1999).

TRAF1, unlike TRAF2 and other TRAFs, does not have the N-terminal RING and zinc-finger domains (Rothe et al., 1994). TRAF1 expression is fairly restricted (Rothe et al., 1994; Mosialos et al., 1995) and can be upregulated in lymphoid tumors and transformed lymphoid cells (Durkop et al., 1999; Zapata et al., 2000). The current data are consistent with the idea that TRAF1 is an NF- κ B inducible protein that protects cells from apoptosis and plays a role in the feedback regulation of receptor signaling (Speiser et al., 1997; Wang et al., 1998; Carpentier and Beyaert, 1999; Schwenzler et al., 1999; Nolan et al., 2000). It appears that TRAF1 works in conjunction with TRAF2 and cIAPs to fully suppress TNF-induced apoptosis. This may be achieved through the direct suppression of caspase activation in the TNFR1 signaling complex by cIAPs, which

led to more specific defects in CD40- and CD27-mediated lymphocyte activation, whereas TNF-mediated NF- κ B activation was not severely affected (Nakano et al., 1999). Interestingly, TRAF2 and 5 double knockout animals did exhibit a severe reduction in TNF-induced NF- κ B activation, which suggests that TRAF5 and TRAF2 are partially functionally redundant (Nakano et al., 2000).

TRAF6 possesses a unique receptor-binding specificity that results in its crucial role as the signaling mediator for both the TNF receptor superfamily and the IL-1R/TLR superfamily. As shown by targeted gene ablation, TRAF6 is functionally important for both TRANCE-R-mediated osteoclast activation and CD40 signaling (Lomaga et al., 1999; Naito et al., 1999; Wong et al., 1999b), even though both CD40 and TRANCE-R can also signal through TRAF2 (Pullen et al., 1998; Wong et al., 1998). In the IL-1R/TLR superfamily, lack of TRAF6 leads to defective signaling by IL-1 and IL-18 as well as hyporesponsiveness to bacterial lipopolysaccharides (LPS), the cell wall component of Gram-negative bacteria, which signals through TLR4 (Lomaga et al., 1999; Naito et al., 1999). These observations place TRAF6 as an important player in innate immunity against pathogens.

The functional divergence of TRAFs appears to correlate well with a proposed evolutionary relationship among TRAFs in mammals and other organisms on the basis of sequence conservation in the TRAF domain and gene structure analysis (Grech et al., 2000) (Fig. 1). In this hypothesis, TRAF4 and TRAF6 precursors appear to have arisen earlier in evolution. We propose that TRAF4 and TRAF6 may be functional descendants of dTRAF1 and dTRAF2, which have been implicated in Toll signal transduction (Zapata et al., 2000; Shen et al., 2001). This argument points to the existence of a yet to be identified TRAF4-interacting receptor. On the other hand, TRAF1, 2, 3 and 5 appear to be more recent siblings in the TRAF family (Grech et al., 2000). This observation is supported by the similar receptor-binding specificity of these four TRAFs towards the TNF receptor superfamily (see below) and the lack of known homologues of these receptors beyond mammals.

Common and distinct signal transduction mechanisms up-stream of TRAFs

Each TRAF protein interacts with and mediates the signal transduction of multiple receptors, and in turn each receptor utilizes multiple TRAFs for specific functions (Arch et al., 1998). There are at least three distinct ways that TRAF proteins can be recruited to and activated by ligand-engaged receptors (Fig. 2A). Members of the TNF receptor superfamily that do not contain intracellular death domains, such as TNFR2 and CD40, recruit TRAFs directly via short sequences in their intracellular tails (Rothe et al., 1994; Cheng et al., 1995; Pullen et al., 1998). Those that contain an intracellular death domain, such as TNFR1, first recruit an adapter protein, TRADD, via a death-domain-death-domain interaction (Hsu et al., 1995). TRADD then serves as a central platform of the TNFR1 signaling complex, which assembles TRAF2 (Hsu et al., 1996b) and RIP (Stanger et al., 1995; Hsu et al., 1996a) for survival signaling, and FADD and caspase-8 for the induction of apoptosis (Hsu et al., 1996b). Members of the IL-1R/TLR superfamily contain a protein interaction module known as the TIR domain (Xu et al.,

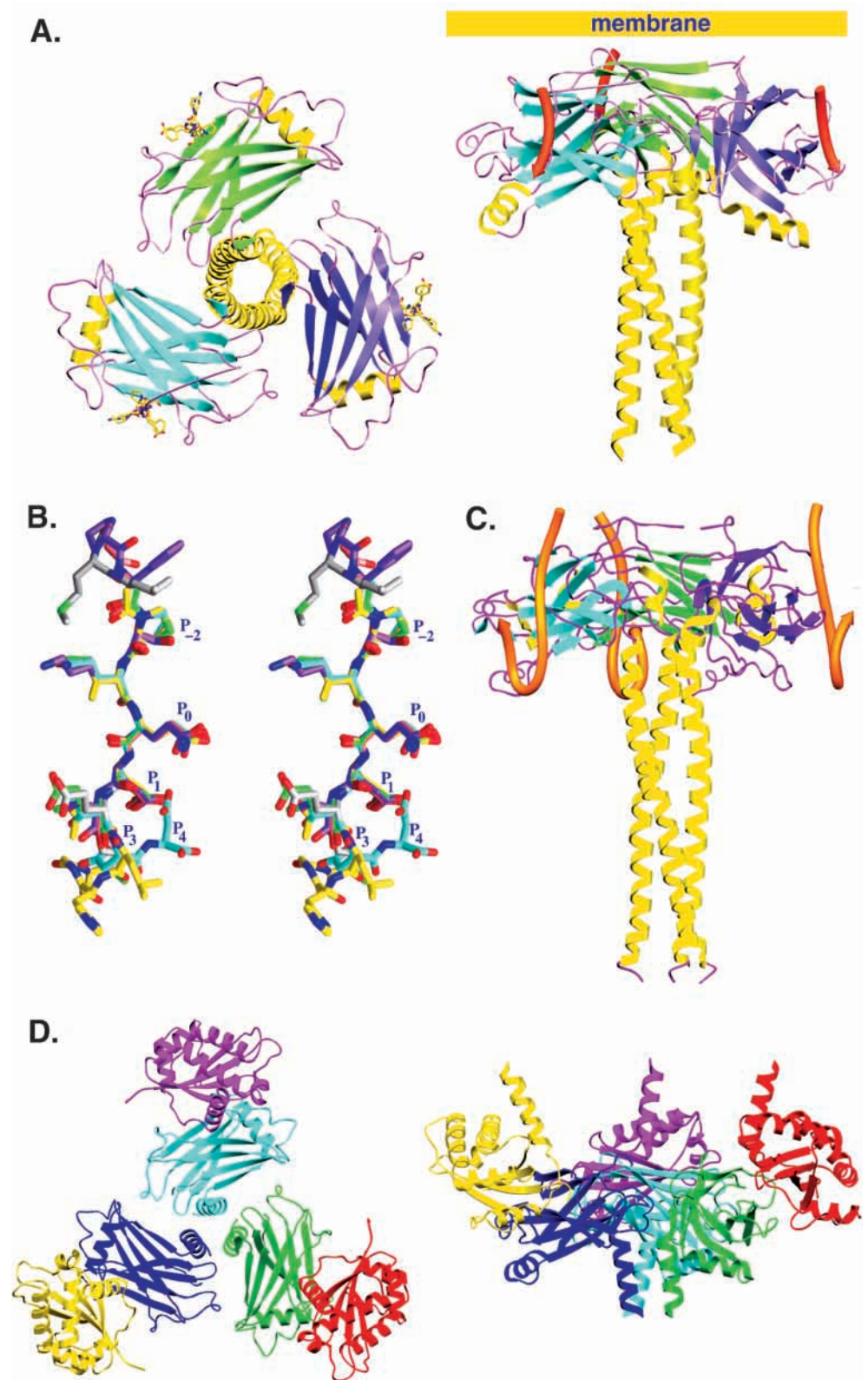
2000), which recruits, sequentially, MyD88 (Wesche et al., 1997), a TIR domain and death domain containing protein, and IRAKs (Cao et al., 1996a; Muzio et al., 1997; Wesche et al., 1999), adapter Ser/Thr kinases with death domains. IRAKs in turn associate with TRAF6 to elicit signaling by IL-1 and pathogenic components such as LPS (Cao et al., 1996b; Zhang et al., 1999; Hacker et al., 2000; Wang et al., 2001).

A common mechanism for the membrane-proximal event in TRAF signaling has been revealed by the conserved trimeric association in the crystal structure of the TRAF domain of TRAF2 (Park et al., 1999; McWhirter et al., 1999). The structure contains a stalk of a trimeric coiled-coil and a cap of trimerized TRAF-C domain with a novel anti-parallel β -sandwich fold, leading to a prominent mushroom shaped structure (Fig. 3A). This trimeric stoichiometry of TRAFs provides a structural basis for signal transduction across the cellular membrane after receptor trimerization by trimeric extracellular ligands in the TNF superfamily (Banner et al., 1993). Interestingly, recent studies suggest that specific ligand-induced receptor trimerization may be primed by non-signaling receptor pre-association prior to ligand binding (Chan et al., 2000; Siegel et al., 2000). Thermodynamic characterization revealed the low affinity nature of monomeric TRAF2-receptor interactions, which confirms the importance of oligomerization-based affinity enhancement or avidity in receptor-mediated TRAF recruitment (Ye and Wu, 2000).

Structural and biochemical studies have shown that a single TRAF protein recognizes diverse receptor sequences via a conserved mode of interaction but with a range of different affinities. In several different TRAF2 complexes, receptor sequences bind invariably to the surface groove on the TRAF-C domain of TRAF2 in an extended conformation, making main chain hydrogen bonding interactions with the edge of the β -sandwich structure (Park et al., 1999; McWhirter et al., 1999; Ye et al., 1999). The chain direction of the receptor peptides allows the receptors to immediately latch on to the TRAF-C domain after exiting from their transmembrane regions. Although TRAF2-binding sequences from different receptors bear limited sequence homology, their interactions with TRAF2 are preserved by a few conserved structural contacts, as shown in the consensus (P/S/T/A)_x(Q/E)E (Ye et al., 1999) (Fig. 3B). A deviation from this consensus, which bears the sequence of PxQxxD, is present in the human Epstein-Barr virus LMP-1 protein and binds to the same surface of TRAF2 via both similar and distinct features (Ye et al., 1999). Thermodynamic characterization further showed variable affinities of TRAF2 with different receptor sequences, which are probably a consequence of affinity modulations by non-conserved residues within and beyond the core binding motif (Ye and Wu, 2000) (Table 3).

Further structural analyses have also revealed how several different TRAFs can recognize a single receptor. The amino-acid residues on the TRAF2 surface used for receptor interactions are conserved among TRAF1, 2, 3 and 5, explaining the overlapping specificity of these TRAFs for different receptors (Park et al., 1999; Ye et al., 1999). However, an identical sequence from CD40 exhibits alternative binding modes to TRAF2 and TRAF3, suggesting that this conserved interaction may vary to some extent in different TRAFs, which modulates the strengths of the interactions (Fig. 3C). In the TRAF3 complex, receptor residues distal to the central core

Fig. 3. Structural studies of upstream interactions of TRAFs. (A) The mushroom-shaped trimeric structure of the TRAF domain of human TRAF2 (left: three-fold axis into the page; right: three-fold axis vertical) is shown here in complex with TNFR2. The coiled-coil region (stalk) is shown as yellow helices. The β -sheet regions of the three TRAF-C domains are shown respectively in blue, green and purple. Bound peptides from TNFR2 are shown as orange arrows, indicating the direction of the peptide chains. The proposed location of the cellular membrane is shown. This figure is modified from (Park et al., 1999). (B) The structural superposition of several TRAF2-interacting receptor peptides is shown using stereo stick models. Nitrogen atoms, blue; oxygen atoms, red; sulfur atoms, green; carbon atoms, yellow (CD40), gray (CD30), green (Ox40), pink (4-1BB), cyan (LMP1) and purple (TNFR2). This figure is adapted from (Ye et al., 1999). (C) The crystal structure of the trimeric complex between the TRAF domain of TRAF3 and a CD40 peptide bound in a hairpin configuration (Ni et al., 2000). The color-coding of the TRAF domain follows that of (A) and the CD40 peptides are shown as orange arrows. (D) A ribbon diagram of the complex between TRADD and TRAF2 (left, three-fold axis into the page; right, three-fold axis vertical). TRAF2, blue, green and purple; TRADD, magenta, red and yellow. The TRAF2-TRADD interface is more extensive and exhibits higher affinity than TRAF2-receptor-peptide interactions. This figure was adapted from Park et al. (Park et al., 2000).



sequence also interact with TRAF3, leading to the formation of a hairpin on the TRAF3 surface, which contributes strongly to TRAF3 interaction (Ni et al., 2000).

The distinct mode of TRAF2 recruitment by TRADD was revealed by the crystal structure of the TRAF2-TRADD

complex (Park et al., 2000) (Fig. 3D). The more extensive TRAF2-TRADD interface overlaps spatially and therefore potentially competes with TRAF2-receptor interactions. Biochemical characterization using surface plasmon resonance has shown that the TRAF2-TRADD interaction is

Table 3. Affinity characterization of the interactions of TRAF2 with various receptor peptides and with TRADD

Receptor peptide	Sequence	K _D (μM)
hCD30 (573-583)	SDVMS SV EEEG	40
hCD40 (250-266)	PV Q ETLHGCGPVT Q EDG	60
hOX40 (262-266)	PI Q EE	50
hTNF-R2 (420-428)	QVPF SK EEC	500
m4-1BB (231-236)	GAA Q EE	1000
hLMP1 (204-210)	P Q QATDD	1900
TRADD		8

The data are from Park et al. (Park et al., 2000) and Ye and Wu (Ye and Wu, 2000). The core sequences are shown in bold and aligned.

unique in two distinct ways. First, TRAF2 has a significantly higher affinity for TRADD than for peptide motifs in direct receptor interactions (Table 3), which leads to more efficient initiation of TRAF2 signaling by TRADD. Second, TRADD has specificity for only TRAF1 and TRAF2, but not other TRAF family members (Fig. 2A). It appears that TRAF1 and TRAF2 work in conjunction with associated caspase inhibitors cIAPs to fully suppress TNF-induced apoptosis in the TNFR1 signaling complex (Wang et al., 1998; Park et al., 2000), leading to dominance of survival signaling for this receptor under most circumstances.

TRAF6 directly interacts with CD40 and TRANCE-R, which are members of the TNF receptor superfamily (Ishida et al., 1996; Pullen et al., 1998; Darnay et al., 1999). For the signal transduction of the IL-1R/TLR superfamily, TRAF6 is indirectly coupled to receptor activation via IRAK and the IRAK-TRAF6 pathway is evolutionarily analogous to the Pelle-dTRAF pathway in *Drosophila* (Liu et al., 1999; Zapata et al., 2000; Shen et al., 2001). Even though biochemical characterizations suggest that TRAF6-receptor and TRAF6-IRAK interactions differ from receptor recognition by other TRAFs (Pullen et al., 1998; Darnay et al., 1999), elucidation of the molecular mechanism of TRAF6 upstream interactions awaits further structural information.

TRAF downstream signal transduction and regulation

TRAF-mediated NF-κB and AP-1 activation has been extensively studied for the representative TRAF family members TRAF2 and TRAF6, which apparently utilize different molecular pathways (Fig. 2B). Two models of TRAF2 downstream signaling pathways have been proposed. The TRAF2-mediated NF-κB activation may involve the direct recruitment of the IKK complex in cooperation with RIP (Yeh et al., 1997; Kelliher et al., 1998; Devin et al., 2000; Nakano et al., 2000; Zhang et al., 2000). Furthermore, artificial oligomerization of either TRAF2 or RIP was sufficient for NF-κB activation (Baud et al., 1999; Poyet et al., 2000). Alternatively, TRAF2 can associate with several upstream MAP kinases to induce NF-κB and AP-1 activation. These include NIK (Malinin et al., 1997; Song et al., 1997), MEKK1 and MEKK3 (Baud et al., 1999; Yang et al., 2001) for IKK activation and ASK1, MEKK1 and GCKR for initiating MAP kinase pathways and AP-1 activation (Nishitoh et al., 1998; Baud et al., 1999; Hoefflich et al., 1999; Shi et al., 1999).

The activation of both NF-κB and AP-1 by TRAF6 in the IL-1 signaling pathway appears to involve a MAP3K known as TAK1 (Yamaguchi et al., 1995; Ninomiya-Tsuji et al., 1999) and two adapter proteins TAB1 (Shibuya et al., 1996) and TAB2 (Takaesu et al., 2000). Upon stimulation, TRAF6 associates with endogenous TAK1 and TAB1 (Ninomiya-Tsuji et al., 1999) and interacts with TAB2 following the translocation of TAB2 from the membrane to the cytosol (Takaesu et al., 2001). Activated TAK1 appears to phosphorylate NIK, which in turn activates IKK (Shirakabe et al., 1997; Ninomiya-Tsuji et al., 1999) and initiates the MAP kinase pathway. Surprisingly, it has been shown recently that ubiquitination plays an important role in TAK1 activation (Deng et al., 2000; Wang et al., 2001). It appears that as a RING-domain-containing protein, TRAF6 operates together with a ubiquitin-conjugating enzyme system to catalyze the synthesis of unique polyubiquitin chains essential for TRAF6 downstream signaling.

The ability of multiple TRAFs to activate NF-κB and AP-1 transcription factors raises the question of how are the specific biological functions of different TRAFs realized. We propose that the different signaling pathways, such as those utilized by TRAF2 and TRAF6, may lead to preferential activation of specific NF-κB and AP-1 components and therefore the transcription of an overlapping but non-identical set of genes. In addition, many TRAF-interacting proteins have been identified and shown to regulate the activation of NF-κB and AP-1 in a TRAF-specific manner. For example, A20 is a TRAF1- and TRAF2-interacting protein (Song et al., 1996) that inhibits NF-κB activation and regulates TNF-induced cell death responses (Lee et al., 2000). A complete review of these regulatory proteins is beyond the scope of this commentary; however, their potential functions should not be overlooked.

A different level of regulation was revealed by several recent gene knockout studies in which certain proteins were shown to regulate NF-κB transcriptional activity without affecting its DNA-binding activity. For example, in mice deficient in the MAP3K NIK, normal NF-κB DNA-binding activity was observed upon treatment by a variety of cytokines, including TNF, IL-1 and LTβ. However, gene transcription upon LTβR activation was selectively affected by the absence of NIK (Yin et al., 2001). Therefore, as different TRAFs may recruit a different set of these regulatory proteins, their biological functions may be modulated by them.

In addition to NF-κB and AP-1 activation, TRAF proteins have been implicated in the crossover to additional signaling pathways. One such example is TRAF6-mediated activation of Src family kinases. In osteoclasts at least, TRAF6 plays an indispensable role in the activation of c-Src and subsequently the anti-apoptotic kinase PKB/Akt (Coffer et al., 1998; Wong et al., 1999a). Similarly, TRAF6-dependent activation of another protein tyrosine kinase Syk has been shown to mediate IL-1-induced chemokine production (Yamada et al., 2001). Therefore, the differential regulation of NF-κB and AP-1, as well as the specific activation of other signaling pathways, may collectively contribute to the specific functions of TRAFs.

Signaling-dependent TRAF trafficking

Accumulating evidence started to identify the intracellular

localization of TRAFs prior to, during and after receptor activation as an important regulatory mechanism for TRAF-mediated signal transduction. In resting cells, several TRAFs have been shown to localize throughout the cytoplasm or to intracellular punctate structures (Mosialos et al., 1995; Hostager et al., 2000). Upon receptor stimulation, TRAFs are redistributed to the cytoplasmic membrane or to plasma membrane patches or caps (Mosialos et al., 1995; Kuhne et al., 1997). More specifically, receptor recruitment of TRAFs during CD40 signaling could lead to the partitioning of these TRAFs into membrane rafts, which are specific regions of the plasma membrane that are rich in sphingolipid and cholesterol (Hostager et al., 2000; Vidalain et al., 2000). This partitioning could be crucial for TRAF signaling as it physically stabilizes the receptor signaling complexes and places TRAFs in the vicinity of a number of signaling proteins including the Src family kinases, which are preferentially localized in these membrane rafts. In fact, it has been found that among the known TRAFs, the ability to redistribute to insoluble membrane fractions consistently correlated with JNK activation. In addition, the forced localization of TRAF3 to the cell membrane was sufficient to convert this molecule into an activator of JNK (Dadgostar and Cheng, 2000).

Although the redistribution of TRAFs into membrane fractions may lead to a more sustained signaling of the activated receptor, it could also lead to a depletion of cytoplasmic TRAFs and therefore downregulate subsequent TRAF-dependent signal transduction (Arch et al., 2000). Some TRAFs can accumulate in perinuclear compartments after a particular signaling event (Arch et al., 2000; Force et al., 2000) but the eventual fate of these TRAFs is not clear. One possibility is proteasome-dependent TRAF degradation (Duckett and Thompson, 1997; Brown et al., 2001), which would limit the recycling of TRAFs for further signal transduction. Interestingly, several TRAFs have been shown to interact with proteins of the cytoskeleton and/or of particular membranes. These include the p62 nucleoporin, a component of the nuclear pore central plug (Gamper et al., 2000), the membrane-organizing protein caveolin-1 (Feng et al., 2001), the microtubule-binding protein MIP-T3 (Ling and Goeddel, 2000) and filamin (Leonardi et al., 2000). Clearly, this is an important field that requires further exploration and may hold many of the clues to the specificity of TRAF-mediated signal transduction.

Perspectives

Since the identification of the first two TRAF family members in 1994, it has become clear that different TRAFs exhibit specific biological functions. The membrane-proximal events for initiating differential TRAF signal transduction have been relatively well established from the wealth of structural and functional studies. The biggest challenge ahead is to further elucidate the molecular mechanisms of specific TRAF downstream signal transduction by differential TRAF localization and interactions with various intracellular proteins.

We thank Joseph Aaron and Zheng-gang Liu for critical readings of the manuscript and wish to apologize in advance for possible incomplete citations.

References

- Arch, R. H., Gedrich, R. W. and Thompson, C. B. (1998). Tumor necrosis factor receptor-associated factors (TRAFs) – a family of adapter proteins that regulates life and death. *Genes. Dev.* **12**, 2821-2830.
- Arch, R. H., Gedrich, R. W. and Thompson, C. B. (2000). Translocation of TRAF proteins regulates apoptotic threshold of cells. *Biochem. Biophys. Res. Commun.* **272**, 936-945.
- Baeuerle, P. A. and Baltimore, D. (1996). NF-kappa B: ten years after. *Cell* **87**, 13-20.
- Banner, D. W., D'Arcy, A., Janes, W., Gentz, R., Schoenfeld, H. J., Broger, C., Loetscher, H. and Lesslauer, W. (1993). Crystal structure of the soluble human 55 kd TNF receptor-human TNF beta complex: implications for TNF receptor activation. *Cell* **73**, 431-445.
- Baud, V., Liu, Z. G., Bennett, B., Suzuki, N., Xia, Y. and Karin, M. (1999). Signaling by proinflammatory cytokines: oligomerization of TRAF2 and TRAF6 is sufficient for JNK and IKK activation and target gene induction via an amino-terminal effector domain. *Genes. Dev.* **13**, 1297-1308.
- Beg, A. A. and Baltimore, D. (1996). An essential role for NF-kappaB in preventing TNF-alpha-induced cell death. *Science* **274**, 782-784.
- Brown, K. D., Hostager, B. S. and Bishop, G. A. (2001). Differential signaling and tumor necrosis factor receptor-associated factor (TRAF) degradation mediated by CD40 and the Epstein-Barr virus oncoprotein latent membrane protein 1 (LMP1). *J. Exp. Med.* **193**, 943-954.
- Cao, Z., Henzel, W. J. and Gao, X. (1996a). IRAK: A kinase associated with the interleukin-1 receptor. *Science* **271**, 1128-1131.
- Cao, Z., Xiong, J., Takeuchi, M., Kurama, T. and Goeddel, D. V. (1996b). TRAF6 is a signal transducer for interleukin-1. *Nature* **383**, 443-446.
- Carpentier, I. and Beyaert, R. (1999). TRAF1 is a TNF inducible regulator of NF-kappaB activation. *FEBS Lett.* **460**, 246-250.
- Chan, F. K., Chun, H. J., Zheng, L., Siegel, R. M., Bui, K. L. and Lenardo, M. J. (2000). A domain in TNF receptors that mediates ligand-independent receptor assembly and signaling. *Science* **288**, 2351-2354.
- Cheng, G., Cleary, A. M., Ye, Z. S., Hong, D. I., Lederman, S. and Baltimore, D. (1995). Involvement of CRAF1, a relative of TRAF, in CD40 signaling. *Science* **267**, 1494-1498.
- Coffer, P. J., Jin, J. and Woodgett, J. R. (1998). Protein kinase B (c-Akt): a multifunctional mediator of phosphatidylinositol 3-kinase activation. *Biochem. J.* **335**, 1-13.
- Dadgostar, H. and Cheng, G. (2000). Membrane localization of TRAF 3 enables JNK activation. *J. Biol. Chem.* **275**, 2539-2544.
- Darnay, B. G., Ni, J., Moore, P. A. and Aggarwal, B. B. (1999). Activation of NF-kappaB by RANK requires tumor necrosis factor receptor-associated factor (TRAF) 6 and NF-kappaB-inducing kinase. Identification of a novel TRAF6 interaction motif. *J. Biol. Chem.* **274**, 7724-7731.
- Deng, L., Wang, C., Spencer, E., Yang, L., Braun, A., You, J., Slaughter, C., Pickart, C. and Chen, Z. J. (2000). Activation of the IkappaB kinase complex by TRAF6 requires a dimeric ubiquitin-conjugating enzyme complex and a unique polyubiquitin chain. *Cell* **103**, 351-361.
- Devin, A., Cook, A., Lin, Y., Rodriguez, Y., Kelliher, M. and Liu, Z. (2000). The distinct roles of TRAF2 and RIP in IKK activation by TNF-R1: TRAF2 recruits IKK to TNF-R1 while RIP mediates IKK activation. *Immunity* **12**, 419-429.
- DiDonato, J. A., Hayakawa, M., Rothwarf, D. M., Zandi, E. and Karin, M. (1997). A cytokine-responsive IkappaB kinase that activates the transcription factor NF-kappaB. *Nature* **388**, 548-554.
- Duckett, C. S. and Thompson, C. B. (1997). CD30-dependent degradation of TRAF2: implications for negative regulation of TRAF signaling and the control of cell survival. *Genes. Dev.* **11**, 2810-2821.
- Duckett, C. S., Gedrich, R. W., Gilfillan, M. C. and Thompson, C. B. (1997). Induction of nuclear factor kappaB by the CD30 receptor is mediated by TRAF1 and TRAF2. *Mol. Cell Biol.* **17**, 1535-1542.
- Durkop, H., Foss, H. D., Demel, G., Klotzbach, H., Hahn, C. and Stein, H. (1999). Tumor necrosis factor receptor-associated factor 1 is overexpressed in Reed-Sternberg cells of Hodgkin's disease and Epstein-Barr virus-transformed lymphoid cells. *Blood* **93**, 617-623.
- Feng, X., Gaeta, M. L., Madge, L. A., Yang, J. H., Bradley, J. R. and Pober, J. S. (2001). Caveolin-1 associates with TRAF2 to form a complex that is recruited to tumor necrosis factor receptors. *J. Biol. Chem.* **276**, 8341-8349.
- Force, W. R., Cheung, T. C. and Ware, C. F. (1997). Dominant negative mutants of TRAF3 reveal an important role for the coiled coil domains in cell death signaling by the lymphotoxin-beta receptor. *J. Biol. Chem.* **272**, 30835-30840.
- Force, W. R., Glass, A. A., Benedict, C. A., Cheung, T. C., Lama, J. and Ware, C. F. (2000). Discrete signaling regions in the lymphotoxin-beta

- receptor for tumor necrosis factor receptor-associated factor binding, subcellular localization, and activation of cell death and NF-kappaB pathways. *J. Biol. Chem.* **275**, 11121-11129.
- Gamper, C., van Eynhoven, W. G., Schweiger, E., Mossbacher, M., Koo, B. and Lederman, S.** (2000). TRAF-3 interacts with p62 nucleoporin, a component of the nuclear pore central plug that binds classical NLS-containing import complexes. *Mol. Immunol.* **37**, 73-84.
- Grech, A., Quinn, R., Srinivasan, D., Badoux, X. and Brink, R.** (2000). Complete structural characterisation of the mammalian and *Drosophila* TRAF genes: implications for TRAF evolution and the role of RING finger splice variants. *Mol. Immunol.* **37**, 721-734.
- Hacker, H., Vabulas, R. M., Takeuchi, O., Hoshino, K., Akira, S. and Wagner, H.** (2000). Immune cell activation by bacterial CpG-DNA through myeloid differentiation marker 88 and tumor necrosis factor receptor-associated factor (TRAF)6. *J. Exp. Med.* **192**, 595-600.
- Hoefflich, K. P., Yeh, W. C., Yao, Z., Mak, T. W. and Woodgett, J. R.** (1999). Mediation of TNF receptor-associated factor effector functions by apoptosis signal-regulating kinase-1 (ASK1). *Oncogene* **18**, 5814-5820.
- Hostager, B. S., Catlett, I. M. and Bishop, G. A.** (2000). Recruitment of CD40 and tumor necrosis factor receptor-associated factors 2 and 3 to membrane microdomains during CD40 signaling. *J. Biol. Chem.* **275**, 15392-15398.
- Hsu, H., Huang, J., Shu, H. B., Baichwal, V. and Goeddel, D. V.** (1996a). TNF-dependent recruitment of the protein kinase RIP to the TNF receptor-1 signaling complex. *Immunity* **4**, 387-396.
- Hsu, H., Shu, H.-B., Pan, M.-G. and Goeddel, D. V.** (1996b). TRADD-TRAF2 and TRADD-FADD interactions define two distinct TNF receptor 1 signal transduction pathways. *Cell* **84**, 299-308.
- Hsu, H., Xiong, J. and Goeddel, D. V.** (1995). The TNF receptor 1-associated protein TRADD signals cell death and NF-kB activation. *Cell* **81**, 495-504.
- Imler, J. L. and Hoffmann, J. A.** (2001). Toll receptors in innate immunity. *Trends Cell Biol.* **11**, 304-311.
- Inoue, J., Ishida, T., Tsukamoto, N., Kobayashi, N., Naito, A., Azuma, S. and Yamamoto, T.** (2000). Tumor necrosis factor receptor-associated factor (TRAF) family: adapter proteins that mediate cytokine signaling. *Exp. Cell Res.* **254**, 14-24.
- 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). Identification of TRAF6, a novel tumor necrosis factor receptor-associated factor protein that mediates signaling from an amino-terminal domain of the CD40 cytoplasmic region. *J. Biol. Chem.* **271**, 28745-28748.
- Ishida, T. K., Tojo, T., Aoki, T., Kobayashi, N., Ohishi, T., Watanabe, T., Yamamoto, T. and Inoue, J.** (1996). TRAF5, a novel tumor necrosis factor receptor-associated factor family protein, mediates CD40 signaling. *Proc. Natl. Acad. Sci. USA* **93**, 9437-9442.
- Karin, M.** (1996). The regulation of AP-1 activity by mitogen-activated protein kinases. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **351**, 127-134.
- Kelliher, M. A., Grimm, S., Ishida, Y., Kuo, F., Stanger, B. Z. and Leder, P.** (1998). The death-domain kinase RIP mediates the TNF-induced NF-kB signal. *Immunity* **8**, 297-303.
- Krajewska, M., Krajewski, S., Zapata, J. M., Van Arsdale, T., Gascoyne, R. D., Berern, K., McFadden, D., Shabaik, A., Hugh, J., Reynolds, A., Clevenger, C. V. and Reed, J. C.** (1998). TRAF-4 expression in epithelial progenitor cells. Analysis in normal adult, fetal, and tumor tissues. *Am. J. Pathol.* **152**, 1549-1561.
- Krappmann, D., Hatada, E. N., Tegethoff, S., Li, J., Klippel, A., Giese, K., Baeuerle, P. A. and Scheidereit, C.** (2000). The I kappa B kinase (IKK) complex is tripartite and contains IKK gamma but not IKAP as a regular component. *J. Biol. Chem.* **275**, 29779-29787.
- Kuhne, M. R., Robbins, M., Hambor, J. E., Mackey, M. F., Kosaka, Y., Nishimura, T., Giggley, J. P., Noelle, R. J. and Calderhead, D. M.** (1997). Assembly and regulation of the CD40 receptor complex in human B cells. *J. Exp. Med.* **186**, 337-342.
- Lee, E. G., Boone, D. L., Chai, S., Libby, S. L., Chien, M., Lodolce, J. P. and Ma, A.** (2000). Failure to regulate TNF-induced NF-kappaB and cell death responses in A20-deficient mice. *Science* **289**, 2350-2354.
- Lee, S. Y., Reichlin, A., Santana, A., Sokol, K. A., Nussenzweig, M. C. and Choi, Y.** (1997). TRAF2 is essential for JNK but not NF-kappaB activation and regulates lymphocyte proliferation and survival. *Immunity* **7**, 703-713.
- Lee, S. Y., Kaufman, D. R., Mora, A. L., Santana, A., Boothby, M. and Choi, Y.** (1998). Stimulus-dependent synergism of the antiapoptotic tumor necrosis factor receptor-associated factor 2 (TRAF2) and nuclear factor kappaB pathways. *J. Exp. Med.* **188**, 1381-1384.
- Leonardi, A., Ellinger-Ziegelbauer, H., Franzoso, G., Brown, K. and Siebenlist, U.** (2000). Physical and functional interaction of filamin (actin-binding protein-280) and tumor necrosis factor receptor-associated factor 2. *J. Biol. Chem.* **275**, 271-278.
- Ling, L. and Goeddel, D. V.** (2000). MIP-T3, a novel protein linking tumor necrosis factor receptor-associated factor 3 to the microtubule network. *J. Biol. Chem.* **275**, 23852-23860.
- Liu, Z. G., Hsu, H., Goeddel, D. V. and Karin, M.** (1996). Dissection of TNF receptor 1 effector functions: JNK activation is not linked to apoptosis while NF-kB activation prevents cell death. *Cell* **87**, 565-576.
- Liu, H., Su, Y. C., Becker, E., Treisman, J. and Skolnik, E. Y.** (1999). A *Drosophila* TNF-receptor-associated factor (TRAF) binds the ste20 kinase Misshapen and activates Jun kinase. *Curr. Biol.* **9**, 101-104.
- Locksley, R. M., Killeen, N. and Lenardo, M. J.** (2001). The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell* **104**, 487-501.
- Lomaga, M. A., Yeh, W., Sarosi, I., Duncan, G. S., Furlonger, C., Ho, A., Morony, S., Capparelli, C., Van, G., Kaufman, S. et al.** (1999). TRAF6 deficiency results in osteopetrosis and defective interleukin-1, CD40, and LPS signaling. *Genes Dev.* **13**, 1015-1024.
- Malinin, N. L., Boldin, M. P., Kovalenko, A. V. and Wallach, D.** (1997). MAP3K-related kinase involved in NF-kB induction by TNF, CD95 and IL-1. *Nature* **385**, 540-544.
- Masson, R., Regnier, C. H., Chenard, M. P., Wendling, C., Mattei, M. G., Tomasetto, C. and Rio, M. C.** (1998). Tumor necrosis factor receptor associated factor 4 (TRAF4) expression pattern during mouse development. *Mech. Dev.* **71**, 187-191.
- McWhirter, S. M., Pullen, S. S., Holton, J. M., Crute, J. J., Kehry, M. R. and Alber, T.** (1999). Crystallographic analysis of CD40 recognition and signaling by human TRAF2. *Proc. Natl. Acad. Sci. USA* **96**, 8408-8413.
- Medzhitov, R. and Janeway, C., Jr** (2000). Innate immune recognition: mechanisms and pathways. *Immunol. Rev.* **173**, 89-97.
- Mizushima, S., Fujita, M., Ishida, T., Azuma, S., Kato, K., Hirai, M., Otsuka, M., Yamamoto, T. and Inoue, J.** (1998). Cloning and characterization of a cDNA encoding the human homolog of tumor necrosis factor receptor-associated factor 5 (TRAF5). *Gene* **207**, 135-140.
- Mosialos, G., Birkenbach, M., Yalamanchili, R., VanArsdale, T., Ware, C. and Kieff, E.** (1995). The Epstein-Barr virus transforming protein LMP1 engages signaling proteins for the tumor necrosis factor receptor family. *Cell* **80**, 389-399.
- Muzio, M., Ni, J., Feng, P. and Dixit, V. M.** (1997). IRAK (Pelle) family member IRAK-2 and MyD88 as proximal mediators of IL-1 signaling. *Science* **278**, 1612-1615.
- Naito, A., Azuma, S., Tanaka, S., Miyazaki, T., Takaki, S., Takatsu, K., Nakao, K., Nakamura, K., Katsuki, M., Yamamoto, T. and Inoue, J.** (1999). Severe osteopetrosis, defective interleukin-1 signalling and lymph node organogenesis in TRAF6-deficient mice. *Genes Cells* **4**, 353-362.
- Nakano, H., Oshima, H., Chung, W., Williams-Abbott, L., Ware, C. F., Yagita, H. and Okumura, K.** (1996). TRAF5, an activator of NF-kappaB and putative signal transducer for the lymphotoxin-beta receptor. *J. Biol. Chem.* **271**, 14661-14664.
- Nakano, H., Sakon, S., Koseki, H., Takemori, T., Tada, K., Matsumoto, M., Munechika, E., Sakai, T., Shirasawa, T., Akiba, H. et al.** (1999). Targeted disruption of Traf5 gene causes defects in CD40- and CD27-mediated lymphocyte activation. *Proc. Natl. Acad. Sci. USA* **96**, 9803-9808.
- Nakano, H., Kurosawa, K., Sakon, S., Yagita, H., Yeh, W. C., Mak, T. W. and Okumura, K.** (2000). Impaired TNF-induced NF-kB activation and high sensitivity to TNF-induced cell death in TRAF2- and TRAF5-double deficient mice. *Scand. J. Immunol.* **51** (suppl 1), 71.
- Ni, C. Z., Welsh, K., Leo, E., Chiou, C. K., Wu, H., Reed, J. C. and Ely, K. R.** (2000). Molecular basis for CD40 signaling mediated by TRAF3. *Proc. Natl. Acad. Sci. USA* **97**, 10395-10399.
- Ninomiya-Tsuji, J., Kishimoto, K., Hiyama, A., Inoue, J., Cao, Z. and Matsumoto, K.** (1999). The kinase TAK1 can activate the NIK-I kappaB as well as the MAP kinase cascade in the IL-1 signalling pathway. *Nature* **398**, 252-256.
- Nishitoh, H., Saitoh, M., Mochida, Y., Takeda, K., Nakano, H., Rothe, M., Miyazono, K. and Ichijo, H.** (1998). ASK1 is essential for JNK/SAPK activation by TRAF2. *Mol. Cell* **2**, 389-395.
- Nolan, B., Kim, R., Duffy, A., Sheth, K., De, M., Miller, C., Chari, R. and Bankey, P.** (2000). Inhibited neutrophil apoptosis: proteasome dependent NF-kappaB translocation is required for TRAF-1 synthesis. *Shock* **14**, 290-294.
- Park, Y. C., Burkitt, V., Villa, A. R., Tong, L. and Wu, H.** (1999). Structural

- basis for self-association and receptor recognition of human TRAF2. *Nature* **398**, 533-538.
- Park, Y. C., Ye, H., Hsia, C., Segal, D., Rich, R. L., Liou, H. C., Myszk, D. G. and Wu, H. (2000). A novel mechanism of TRAF signaling revealed by structural and functional analyses of the TRADD-TRAF2 interaction. *Cell* **101**, 777-787.
- Poyet, J. L., Srinivasula, S. M., Lin, J. H., Fernandes-Alnemri, T., Yamaoka, S., Tsichlis, P. N. and Alnemri, E. S. (2000). Activation of the I κ B kinase by RIP via IKK γ /NEMO-mediated oligomerization. *J. Biol. Chem.* **275**, 37966-37977.
- Preiss, A., Johannes, B., Nagel, A. C., Maier, D., Peters, N. and Wajant, H. (2001). Dynamic expression of *Drosophila* TRAF1 during embryogenesis and larval development. *Mech. Dev.* **100**, 109-113.
- Pullen, S. S., Miller, H. G., Everdeen, D. S., Dang, T. T., Crute, J. J. and Kehry, M. R. (1998). CD40-tumor necrosis factor receptor-associated factor (TRAF) interactions: regulation of CD40 signaling through multiple TRAF binding sites and TRAF hetero-oligomerization. *Biochemistry* **37**, 11836-11845.
- Regnier, C. H., Tomasetto, C., Moog-Lutz, C., Chenard, M. P., Wendling, C., Basset, P. and Rio, M. C. (1995). Presence of a new conserved domain in CART1, a novel member of the tumor necrosis factor receptor-associated protein family, which is expressed in breast carcinoma. *J. Biol. Chem.* **270**, 25715-25721.
- Regnier, C. H., Song, H. Y., Gao, X., Goeddel, D. V., Cao, Z. and Rothe, M. (1997). Identification and characterization of an I κ B kinase. *Cell* **90**, 373-383.
- Reinhard, C., Shamoan, B., Shyamala, V. and Williams, L. T. (1997). Tumor necrosis factor alpha-induced activation of c-jun N-terminal kinase is mediated by TRAF2. *EMBO J.* **16**, 1080-1092.
- Rothe, M., Wong, S. C., Henzel, W. J. and Goeddel, D. V. (1994). A novel family of putative signal transducers associated with the cytoplasmic domain of the 75 kDa tumor necrosis factor receptor. *Cell* **78**, 681-692.
- Rothe, M., Sarma, V., Dixit, V. M. and Goeddel, D. V. (1995). TRAF2-mediated activation of NF- κ B by TNF receptor 2 and CD40. *Science* **269**, 1424-1427.
- Sato, T., Irie, S. and Reed, J. C. (1995). A novel member of the TRAF family of putative signal transducing proteins binds to the cytosolic domain of CD40. *FEBS Lett.* **358**, 113-118.
- Schwenzer, R., Siemiński, K., Liptay, S., Schubert, G., Peters, N., Scheurich, P., Schmid, R. M. and Wajant, H. (1999). The human tumor necrosis factor (TNF) receptor-associated factor 1 gene (TRAF1) is up-regulated by cytokines of the TNF ligand family and modulates TNF-induced activation of NF- κ B and c-Jun N-terminal kinase. *J. Biol. Chem.* **274**, 19368-19374.
- Shaulian, E. and Karin, M. (2001). AP-1 in cell proliferation and survival. *Oncogene* **20**, 2390-2400.
- Shen, B., Liu, H., Skolnik, E. Y. and Manley, J. L. (2001). Physical and functional interactions between *Drosophila* TRAF2 and Pelle kinase contribute to Dorsal activation. *Proc. Natl. Acad. Sci. USA* **98**, 8596-8601.
- Shi, C. S., Leonardi, A., Kyriakis, J., Siebenlist, U. and Kehrl, J. H. (1999). TNF-mediated activation of the stress-activated protein kinase pathway: TNF receptor-associated factor 2 recruits and activates germinal center kinase related. *J. Immunol.* **163**, 3279-3285.
- Shibuya, H., Yamaguchi, K., Shirakabe, K., Tonegawa, A., Gotoh, Y., Ueno, N., Irie, K., Nishida, E. and Matsumoto, K. (1996). TAB1: an activator of the TAK1 MAPKKK in TGF- β signal transduction. *Science* **272**, 1179-1182.
- Shiels, H., Li, X., Schumacker, P. T., Maltepe, E., Padrid, P. A., Sperling, A., Thompson, C. B. and Lindsten, T. (2000). TRAF4 deficiency leads to tracheal malformation with resulting alterations in air flow to the lungs. *Am. J. Pathol.* **157**, 679-688.
- Shirakabe, K., Yamaguchi, K., Shibuya, H., Irie, K., Matsuda, S., Moriguchi, T., Gotoh, Y., Matsumoto, K. and Nishida, E. (1997). TAK1 mediates the ceramide signaling to stress-activated protein kinase/c-Jun N-terminal kinase. *J. Biol. Chem.* **272**, 8141-8144.
- Siegel, R. M., Frederiksen, J. K., Zacharias, D. A., Chan, F. K., Johnson, M., Lynch, D., Tsien, R. Y. and Lenardo, M. J. (2000). Fas preassociation required for apoptosis signaling and dominant inhibition by pathogenic mutations. *Science* **288**, 2354-2357.
- Song, H. Y., Rothe, M. and Goeddel, D. V. (1996). The tumor necrosis factor-inducible zinc finger protein A20 interacts with TRAF1/TRAF2 and inhibits NF- κ B activation. *Proc. Natl. Acad. Sci. USA* **93**, 6721-6725.
- Song, H. Y., Regnier, C. H., Kirschning, C. J., Goeddel, D. V. and Rothe, M. (1997). Tumor necrosis factor (TNF)-mediated kinase cascades: bifurcation of nuclear factor- κ B and c-jun N-terminal kinase (JNK/SAPK) pathways at TNF receptor-associated factor 2. *Proc. Natl. Acad. Sci. USA* **94**, 9792-9796.
- Speiser, D. E., Lee, S. Y., Wong, B., Arron, J., Santana, A., Kong, Y. Y., Ohashi, P. S. and Choi, Y. (1997). A regulatory role for TRAF1 in antigen-induced apoptosis of T cells. *J. Exp. Med.* **185**, 1777-1783.
- Stancovski, I. and Baltimore, D. (1997). NF- κ B activation: The I κ B kinase revealed? *Cell* **91**, 299-302.
- Stanger, B. Z., Leder, P., Lee, T., Kim, E. and Seed, B. (1995). RIP: a novel protein containing a death domain that interacts with Fas/APO-1 (CD95) in yeast and causes cell death. *Cell* **81**, 513-523.
- Takaesu, G., Kishida, S., Hiyama, A., Yamaguchi, K., Shibuya, H., Irie, K., Ninomiya-Tsuji, J. and Matsumoto, K. (2000). TAB2, a novel adaptor protein, mediates activation of TAK1 MAPKKK by linking TAK1 to TRAF6 in the IL-1 signal transduction pathway. *Mol. Cell* **5**, 649-658.
- Takaesu, G., Ninomiya-Tsuji, J., Kishida, S., Li, X., Stark, G. R. and Matsumoto, K. (2001). Interleukin-1 (IL-1) receptor-associated kinase leads to activation of TAK1 by inducing TAB2 translocation in the IL-1 signaling pathway. *Mol. Cell Biol.* **21**, 2475-2484.
- Takaori-Kondo, A., Hori, T., Fukunaga, K., Morita, R., Kawamata, S. and Uchiyama, T. (2000). Both amino- and carboxyl-terminal domains of TRAF3 negatively regulate NF- κ B activation induced by OX40 signaling. *Biochem. Biophys. Res. Commun.* **272**, 856-863.
- Takeuchi, M., Rothe, M. and Goeddel, D. V. (1996). Anatomy of TRAF2. Distinct domains for nuclear factor- κ B activation and association with tumor necrosis factor signaling proteins. *J. Biol. Chem.* **271**, 19935-19942.
- van Eynhoven, W. G., Gamper, C. J., Cho, E., Mackus, W. J. and Lederman, S. (1999). TRAF-3 mRNA splice-deletion variants encode isoforms that induce NF- κ B activation. *Mol. Immunol.* **36**, 647-658.
- Vidalain, P. O., Azocar, O., Servet-Delprat, C., Rabourdin-Combe, C., Gerlier, D. and Manie, S. (2000). CD40 signaling in human dendritic cells is initiated within membrane rafts. *EMBO J.* **19**, 3304-3313.
- Wallach, D., Varfolomeev, E. E., Malinin, N. L., Goltsev, Y. V., Kovalenko, A. V. and Boldin, M. P. (1999). Tumor necrosis factor receptor and Fas signaling mechanisms. *Annu. Rev. Immunol.* **17**, 331-367.
- Wajant, H., Muhlenbeck, F. and Scheurich, P. (1998). Identification of a TRAF (TNF receptor-associated factor) gene in *Caenorhabditis elegans*. *J. Mol. Evol.* **47**, 656-662.
- Wajant, H., Henkler, F. and Scheurich, P. (2001). The TNF-receptor-associated factor family: scaffold molecules for cytokine receptors, kinases and their regulators. *Cell Signal.* **13**, 389-400.
- Wang, C., Deng, L., Hong, M., Akkaraju, G. R., Inoue, J. and Chen, Z. J. (2001). TAK1 is a ubiquitin-dependent kinase of MKK and IKK. *Nature* **412**, 346-351.
- Wang, C. Y., Mayo, M. W., Korneluk, R. G., Goeddel, D. V. and Baldwin, A. S., Jr (1998). NF- κ B antiapoptosis: induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. *Science* **281**, 1680-1683.
- Wang, Q., Dziarski, R., Kirschning, C. J., Muzio, M. and Gupta, D. (2001). Micrococci and peptidoglycan activate TLR2 \rightarrow MyD88 \rightarrow IRAK \rightarrow TRAF \rightarrow NIK \rightarrow IKK \rightarrow NF- κ B signal transduction pathway that induces transcription of interleukin-8. *Infect. Immun.* **69**, 2270-2276.
- Wesche, H., Henzel, W. J., Shillinglaw, W., Li, S. and Cao, Z. (1997). MyD88: an adapter that recruits IRAK to the IL-1 receptor complex. *Immunity* **7**, 837-847.
- Wesche, H., Gao, X., Li, X., Kirschning, C. J., Stark, G. R. and Cao, Z. (1999). IRAK-M is a novel member of the Pelle/interleukin-1 receptor-associated kinase (IRAK) family. *J. Biol. Chem.* **274**, 19403-19410.
- Wong, B. R., Josien, R., Lee, S. Y., Volododskaia, M., Steinman, R. M. and Choi, Y. (1998). The TRAF family of signal transducers mediates NF- κ B activation by the TRANCE receptor. *J. Biol. Chem.* **273**, 28355-28359.
- Wong, B. R., Besser, D., Kim, N., Arron, J. R., Volododskaia, M., Hanafusa, H. and Choi, Y. (1999). TRANCE, a TNF family member, activates Akt/PKB through a signaling complex involving TRAF6 and c-Src. *Mol. Cell* **4**, 1041-1049.
- Wong, B. R., Josien, R. and Choi, Y. (1999). TRANCE is a TNF family member that regulates dendritic cell and osteoclast function. *J. Leukoc. Biol.* **65**, 715-724.
- Xu, Y., Cheng, G. and Baltimore, D. (1996). Targeted disruption of TRAF3 leads to postnatal lethality and defective T-dependent immune responses. *Immunity* **5**, 407-415.
- Xu, Y., Tao, X., Shen, B., Horng, T., Medzhitov, R., Manley, J. L. and Tong,

- L. (2000). Structural basis for signal transduction by the Toll/interleukin-1 receptor domains. *Nature* **408**, 111-115.
- Yamada, T., Fujieda, S., Yanagi, S., Yamamura, H., Inatome, R., Yamamoto, H., Igawa, H. and Saito, H.** (2001). Il-1 induced chemokine production through the association of syk with tnf receptor-associated factor-6 in nasal fibroblast lines. *J Immunol* **167**, 283-288.
- Yamaguchi, K., Shirakabe, K., Shibuya, H., Irie, K., Oishi, I., Ueno, N., Taniguchi, T., Nishida, E. and Matsumoto, K.** (1995). Identification of a member of the MAPKKK family as a potential mediator of TGF-beta signal transduction. *Science* **270**, 2008-2011.
- Yang, J., Lin, Y., Guo, Z., Cheng, J., Huang, J., Deng, L., Liao, W., Chen, Z., Liu, Z. and Su, B.** (2001). The essential role of MEKK3 in TNF-induced NF-kappaB activation. *Nat. Immunol.* **2**, 620-624.
- Ye, H., Park, Y. C., Kreishman, M., Kieff, E. and Wu, H.** (1999). The structural basis for the recognition of diverse receptor sequences by TRAF2. *Mol. Cell* **4**, 321-330.
- Ye, H. and Wu, H.** (2000). Thermodynamic characterization of the interaction between TRAF2 and receptor peptides by isothermal titration calorimetry. *Proc. Natl. Acad. Sci. USA* **97**, 8961-8966.
- Ye, X., Mehlen, P., Rabizadeh, S., VanArsdale, T., Zhang, H., Shin, H., Wang, J. J., Leo, E., Zapata, J., Hauser, C. A., Reed, J. C. and Bredesen, D. E.** (1999). TRAF family proteins interact with the common neurotrophin receptor and modulate apoptosis induction. *J. Biol. Chem.* **274**, 30202-30208.
- Yeh, W. C., Shahinian, A., Speiser, D., Kraunus, J., Billia, F., Wakeham, A., de la Pompa, J. L., Ferrick, D., Hum, B., Iscove, N., Ohashi, P., Rothe, M., Goeddel, D. V. and Mak, T. W.** (1997). Early lethality, functional NF-kappaB activation, and increased sensitivity to TNF-induced cell death in TRAF2-deficient mice. *Immunity* **7**, 715-725.
- Yeh, W. C., Hakem, R., Woo, M. and Mak, T. W.** (1999). Gene targeting in the analysis of mammalian apoptosis and TNF receptor superfamily signaling. *Immunol. Rev.* **169**, 283-302.
- Yin, L., Wu, L., Wesche, H., Arthur, C. D., White, J. M., Goeddel, D. V. and Schreiber, R. D.** (2001). Defective lymphotoxin-beta receptor-induced NF-kappaB transcriptional activity in NIK-deficient mice. *Science* **291**, 2162-2165.
- Zandi, E., Rothwarf, D. M., Delhase, M., Hayakawa, M. and Karin, M.** (1997). The IkappaB kinase complex (IKK) contains two kinase subunits, IKKalpha and IKKbeta, necessary for IkappaB phosphorylation and NF-kappaB activation. *Cell* **91**, 243-252.
- Zapata, J. M., Krajewska, M., Krajewski, S., Kitada, S., Welsh, K., Monks, A., McCloskey, N., Gordon, J., Kipps, T. J., Gascoyne, R. D., Shabaik, A. and Reed, J. C.** (2000). TNFR-associated factor family protein expression in normal tissues and lymphoid malignancies. *J. Immunol.* **165**, 5084-5096.
- Zapata, J. M., Matsuzawa, S., Godzik, A., Leo, E., Wasserman, S. A. and Reed, J. C.** (2000). The *Drosophila* tumor necrosis factor receptor-associated factor-1 (DTRAF1) interacts with Pelle and regulates NFKappaB activity. *J. Biol. Chem.* **275**, 12102-12107.
- Zhang, F. X., Kirschning, C. J., Mancinelli, R., Xu, X. P., Jin, Y., Faure, E., Mantovani, A., Rothe, M., Muzio, M. and Arditi, M.** (1999). Bacterial lipopolysaccharide activates nuclear factor-kappaB through interleukin-1 signaling mediators in cultured human dermal endothelial cells and mononuclear phagocytes. *J. Biol. Chem.* **274**, 7611-7614.
- Zhang, S. Q., Kovalenko, A., Cantarella, G. and Wallach, D.** (2000). Recruitment of the IKK signalosome to the p55 TNF receptor: RIP and A20 bind to NEMO (IKKgamma) upon receptor stimulation. *Immunity* **12**, 301-311.