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# Structural Basis of Signal Transduction in the TNF Receptor Superfamily

**Jixi Li**, **Qian Yin**, and **Hao Wu**[1]
Program in Cellular and Molecular Medicine, Boston Children's Hospital and Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts, USA

## Abstract

Members of the tumor necrosis factor receptor superfamily play key roles in innate and adaptive immunity. Here, we review recent structural studies in the intracellular signal transduction of these receptors. A central theme revealed from these structural studies is that upon ligand binding, multiple intracellular proteins form higher-order signaling machines to transduce and amplify receptor activation information to different cellular fates, including NF-κB activation, apoptosis, and programmed necrosis. These studies open a new vista for understanding the biophysical principles in these signaling cascades.

## 1. INTRODUCTION

The tumor necrosis factor receptor (TNFR) superfamily consists of 29 transmembrane receptors. Members of TNFRs contain an extracellular domain responsible for ligand binding and an intracellular domain that mediates activation of signaling pathway (Aggarwal, 2003; Bodmer, Schneider, & Tschopp, 2002; Locksley, Killeen, & Lenardo, 2001). TNFRs may be divided into two groups: activating receptors and death receptors (DRs). Most TNFRs are activating receptors, such as CD40 and TNFR2, which can activate nuclear factor κB (NF-κB) and mitogen-activated protein kinase (MAPK) pathways. DRs include eight members, such as TNFR1 and Fas, which have a protein interaction module called the death domain (DD) in the intracellular region that mediates extrinsic signal-induced cell death (Wu & Hymowitz, 2009). TNFR1 is a pleiotropic receptor and is able to induce both activating and death signaling pathways to effect cell metabolism, differentiation, and proliferation (Moquin & Chan, 2010; Schröfelbauer & Hoffmann, 2011). It is activated by the ligand TNFα, which is the founding member of the TNF superfamily.

The ligand/receptor interaction at the extracellular domain has been first revealed by the crystal structure of the trimeric TNFβ-bound symmetrically to the extracellular region of three TNFR1 molecules (Banner et al., 1993). Each TNFR1 chain contacts the interfaces between two protomers of a TNF trimer (Wu & Hymowitz, 2009). A number of subsequent structures of ligand/receptor complexes further confirmed the 3:3 symmetrical interactions at the extracellular region. In this review, we focus on the intra-cellular events in TNFR signaling. Inparticular, we illustrate the structural basis for the induction of NF-κB activation, apoptosis, and programmed necrosis.



[1]Corresponding author: hao.wu@childrens.harvard.edu.

## 2. NF-κB ACTIVATION

Members of the TNFR superfamily activate NF-κB in two alternatively pathways, exemplified by TNFR1 and CD40, respectively. Upon binding with TNFα, the intracellular DD of TNFR1 recruits TNF receptor-associated DD protein (TRADD), which in turn recruits receptor-interacting protein kinase 1 (RIP1), cellular inhibitor of apoptosis proteins 1 and 2 (cIAP1 and 2), and TNF receptor-associated factor 2 (TRAF2; Fig. 5.1). TRADD is important for the TNF-induced NF-κB signaling pathway, as in TRADD-deficient MEFs, IκB phosphorylation and degradation are completely abolished (Chen et al., 2008). The N-terminal region of TRADD interacts with the trimeric TRAF domain of TRAF2 in a 3:3 stoichiometry, whereas the C-terminal DD-containing region of TRADD interacts with many other DD-containing proteins, such as FADD and RIP1 (Park et al., 2000).

The inhibitor of apoptosis proteins cIAP1 and cIAP2 acts as an E3 ligase to form K63 polyubiquitin chains on RIP1 and itself, providing a platform for recruitment of NEMO, the regulatory subunit of the IKK complex (Mahoney et al., 2008). Meanwhile, cIAP1 together with E2 UbcH5 can generate K11 polyubiquitin chains on RIP1 within the endogenous TNFR1 complex and activate NF-κB (Dynek et al., 2010). cIAPs consist of two parts: the N-terminal three baculoviral IAP repeats (BIRs) and CARD and RING domains at the C-terminal region. The structures of BIR1/3 domains, CARD, and RING domains have been determined (Lopez et al., 2011; Mace et al., 2008; Zheng, Kabaleeswaran, Wang, Cheng, & Wu, 2010).

RIP1 is a key factor in mediating TNF-induced signal pathways. In RIP1-deficient T and B cells, TNF-induced NF-κB activation was totally abolished (Feltham et al., 2010). When the E3 ligases TRAF2/cIAP and linear ubiquitin chain assembly complex (LUBAC) ubiquitinate RIP1 in the TNFR1 signaling complex, polyubiquitinated RIP1 engages downstream adaptors such as TGF beta-activated kinase 1 (TAK1) and NEMO to activate IKK, promoting NF-κB transcriptional activity, and leading to cell survival, proliferation, and differentiation (Walczak, 2011).

Besides K63 polyubiquitination, RIP1 and NEMO can also be modified with linear polyubiquitin chain, which is executed by LUBAC, consisting of HOIL-1, HOIP, and SHARPIN (Gerlach et al., 2011; Ikeda et al., 2011). LUBAC can increase the recruitment of cIAP1/2, TRAF2, RIP1, and TAK1 among the TNFR signaling complex, and the depletion of any LUBAC component decreases NF-κB and MAPK activation (Haas et al., 2009).

In the CD40-mediated NF-κB pathway, TRAF6 directly interacts with the intracellular region of the receptor and acts as the ubiquitin ligase to induced K63-linked polyubiquitination (Deng et al., 2000; Fig. 5.1). Similar to the TNFR1 pathway, the polyubiquitin chains engage downstream signaling proteins such as TAK1 and NEMO to activate IKK, leading to IκB phosphorylation, nuclear translocation of NF-κB, and transcription of NF-κB-controlled genes for cell survival, proliferation, and differentiation.

### 2.1. Structures of TRAFs and TAK1 complex

TRAF proteins consist of two parts: an N-terminal RING/zinc-finger domain and a C-terminal coiled-coil/TRAF-C domain (Fig. 5.2A). The N-terminal region of TRAF6 functions as an ubiquitin E3 ligase for K63-linked polyubiquitination (Yin, Lamothe, Darnay, & Wu, 2009; Yin, Lin, et al., 2009). In the TNFR1 pathway, cIAP1 and cIAP2 are the analogous E3 ligases. The coiled-coil/TRAF-C domain mediates interactions with receptors and adaptor proteins, such as TNFR2, CD30, CD40, and TRADD, and is responsible for the specificity and diversity of TRAF recruitment (Park, Burkitt, Villa, Tong, & Wu, 1999; Park et al., 2000; Ye, Cirilli, & Wu, 2002; Ye, Park, Kreishman, Kieff, & Wu,

1999; Ye & Wu, 2000; Zheng et al., 2010). TRAF2 can be recruited to TNFRs either via direct interactions or via intermediate adapter proteins such as TRADD, and TRAF6 is recruited directly to CD40. The interaction between TRADD and TRAF2 (Fig. 5.2B) is much stronger than that in receptor-TRAF2, which ensures the downstream cIAPs recruitment for the direct inhibition of caspase activation in the signaling complex (Park et al., 2000). The trimeric coiled-coil domain of TRAF2 forms a complex with one cIAP2 via direct interaction from two TRAF2 chains (Zheng et al., 2010; Fig. 5.2C).

Unexpectedly, the N-terminal RING/ZF domains of TRAF6 form a dimer in solution and in the crystal (Yin, Lamothe, et al., 2009; Yin, Lin, et al., 2009; Fig. 5.2D). Dimerization of TRAF6 is important for its E3 ligase activity, which promotes the assembly of polyubiquitination and IκB phosphorylation (Yin, Lamothe, et al., 2009; Yin, Lin, et al., 2009). Based on the symmetry mismatch between the dimeric N-terminal region and the trimeric C-terminal region, a 2D lattice model was proposed to elucidate the infinite oligomerization of TRAF6 and other TRAFs (Fig. 5.2E).

The kinase complex TAK1, also called MAP3K7 and MEKK7 (Yamaguchi et al., 1995), is composed of TAK1 and TAK1-binding proteins (TAB1/2/3; Ishitani et al., 2003). In the TAK1/TAB1 complex structure, the C-terminal lobe of TAK1 kinase domain (KD) forms an extensive interface with an α helix of TAB1 (Brown et al., 2005; Fig. 5.2F). This interaction promotes TAK1 autophosphorylation, most likely through an allosteric mechanism (Ono et al., 2001; Sakurai, Miyoshi, Mizukami, & Sugita, 2000). TAB2 and TAB3 facilitate TAK1 activation via recruiting the K63-linked polyubiquitin chains. The N-terminal zinc-finger domain of TAB2/TAB3 binds diubiquitin identically in crystal structures, and prefers the K63-linked polyubiquitin chains to K48-linked ones as the conformational constraint does not favor the linear linkage (Ono et al., 2001; Sakurai et al., 2000; Fig. 5.2G). The TRAF-polyubiquitin chains act as a scaffold to bring the activated TAK1 to the proximal space of IKK and, in turn, phosphorylate and activate IKK complex.

### 2.2. Structure of the IKK complex

The IKK complex consists of two catalytic subunits IKKα/IKKβ and a regulatory subunit NEMO (IKKg; Chen, Parent, & Maniatis, 1996; Mercurio et al., 1997). The recently solved IKKβ structure reveals a trimodular architecture (Xu et al., 2011; Fig. 5.3A and B), which is composed of a KD, an ubiquitin-like domain (ULD), and an elongated α-helical scaffold/dimerization domain (SDD). IKKβ is dimeric in solution and in crystal lattice (Xu et al., 2011); however, IKKβ may form a high-order oligomerization during activation as the dimeric conformation does not facilitate the intra-dimeric *trans*-autophosphorylation. NEMO contains a UBAN (ubiquitin binding in ABIN and NEMO) and a zinc-finger (ZF) domain at its C-terminal end, which mediates the interaction between the IKK complex and the polyubiquitin chains (Rothwarf, Zandi, Natoli, & Karin, 1998; Yamaoka et al., 1998). The UBAN domain prefers linear poly-ubiquitin chains, evidenced in the crystal structures that UBAN binds to both ubiquitins in linear diubiquitin but does not make simultaneous contacts with both ubiquitins in K63-linked diubiquitin (Lo et al., 2009; Rahighi et al., 2009; Yoshikawa et al., 2009; Fig. 5.3C). Most likely, linear and K63-linked polyubiquitin chains play roles in response to different stimuli. NEMO binds to the C-terminal NEMO-binding domain (NBD) of IKKα and IKKβ (Marienfeld, Palkowitsch, & Ghosh, 2006) and forms a parallel four-helix bundled heterotetramer with two molecules of each protein (Rushe et al., 2008). Based on the structural information available for IKKβ and NEMO, a model for the IKK complex was proposed (Ferrao, Li, Bergamin, & Wu, 2012; Zheng, Yin, & Wu, 2011; Fig. 5.3C).

## 3. TNFR1 AND FAS-INDUCED APOPTOSIS

TNFR1 and Fas belong to the DR family. Fas induces cell apoptotic death. When the ligand FasL binds to the extracellular region of Fas, the cytosolic region of Fas recruits the adaptor protein FADD via the DD interaction. FADD consists of a DD and a death effector domain (DED), through which FADD recruits caspase-8 and -10 via the interactions with the tandem DEDs in the prodomain of the caspases (Carrington et al., 2006; Strasser, Jost, & Nagata, 2009; Fig. 5.4A). The ternary complex, composed with Fas, FADD, and caspase-8/10, has been traditionally named the death-inducing signaling complex (DISC; Kischkel et al., 1995), which brings the catalytic domains of the caspases into proximity for dimerization and autoprocessing. DDs and DEDs belong to the DD-fold superfamily (Park et al., 2007). Proteins containing these domains form oligomeric complexes through homotypic interactions, which play central roles in different apoptotic and inflammatory pathways (Ferrao & Wu, 2012).

Upon ligand binding, the cytosolic region of TNFR1 forms a large complex, which includes TRADD, TRAF2/5, cIAP1/2, and RIP1. When K63-linked polyubiquitin chain of RIP1 is removed by the deubiquitinases CYLD (cylindromatosis) or A20 (Sun, 2010; Wilson, Dixit, & Ashkenazi, 2009), or blocked by removal of the E3 ligases cIAP1 and cIAP2 through genetic ablation, RNAi knockdown, or IAP antagonists, RIP1 and its family member RIP3 are recruited to a second complex containing TRADD, FADD, and caspase-8 (Feoktistova et al., 2011; Tenev et al., 2011). In either the TRADD/FADD/caspase-8 or the RIP1/FADD/caspase-8 complex, caspase-8 is activated, which in turn cleaves RIP1 and RIP3, and induces cell apoptosis (Bertrand et al., 2008; Wang, Du, & Wang, 2008).

### 3.1. Structures of individual proteins in the DISC

The NMR structure of Fas DD reveals an antiparallel six-helical bundle architecture, which is common among the entire DD superfamily (Huang, Eberstadt, Olejniczak, Meadows, & Fesik, 1996). The DD, DED, and full-length (FL) FADD structures have been determined by NMR method (Carrington et al., 2006; Eberstadt et al., 1998; Jeong et al., 1999). The structure of the tandem DED domain of caspase-8 or caspase-10 has not been resolved. However, the structure of the tandem DED domain of a viral caspase-8/10 and FLICE/caspase-8 inhibitory protein (FLIP) from poxvirus *Molluscum contagiosum* virus has been determined, revealing a dumbbell-shaped arrangement common to all tandem DEDs (Li, Jeffrey, Yu, & Shi, 2006; Yang et al., 2005; Fig. 5.4B). The structure of the catalytic domain of caspase-8 reveals a dimeric structure common to all caspases (Blanchard et al., 1999; Watt et al., 1999; Fig. 5.4C).

### 3.2. Structure of the Fas DD:FADD DD complex in the DISC

The structure of the Fas DD:FADD DD complex has been elucidated (Wang et al., 2010; Fig. 5.4D). Similar to the structure of PIDD DD: RAIDD DD complex (Park et al., 2007), the class projection averages of negatively stained Fas DD:FADD DD complex shows an asymmetric oligomeric structure. Moreover, it was shown that the Fas DD:FADD DD complex contains a mixture of 5 Fas:5 FADD, 6 Fas:5 FADD, and 7 Fas:5 FADD complexes evidenced by the nanoflow electrospray ionization and tandem mass spectrometry method. Based on the layered structure of the PIDD DD:RAIDD DD complex, a 5:5 core Fas DD:FADD DD complex was built and used to solve a low-resolution crystal structure of the complex (Wang et al., 2010). Mutations on the interfaces between Fas and FADD affected the complex formation and directly explained the dominant-negative effects from Fas mutations that are associated with autoimmune lymphoproliferative syndrome in humans (Wang et al., 2010). The structure shows the similar helical assembly architecture as the death domain complex in Toll-like receptor signaling (Lin et al., 2010).

### 3.3. DED chains in the DISC

The formation of the DISC is essential for Fas-mediated apoptosis. Recent findings identified the stoichiometry of the Fas DISC (Dickens et al., 2012; Schleich et al., 2012). Among the DISC, the amount of DED proteins procaspase-8/10 and c-FLIP exceeds that of FADD by seven- to ninefold with quantitative western blots, mass spectrometry, and mathematical modeling methods (Dickens et al., 2012; Schleich et al., 2012). One proposed model showed that procaspase-8/10 and c-FLIP could form a caspase-activating chain via their DED domains. Mutations of some key interacting residues in procaspase-8 DED2 abrogate DED chain formation in cells (Dickens et al., 2012; Schleich et al., 2012). Moreover, the DED of FADD and the DED2 of procaspase-8 form filaments in Hela and Jurkat Tag cells, which can be blocked by coexpression of viral antiapoptotic DED-containing proteins (MC159 and E8), but not by bcl-2 family proteins (Siegel et al., 1998). The DED chain assembly in DISC may drive caspase-8 dimerization and activation, leading to cell apoptosis.

## 4. TNFR1-INDUCED PROGRAMMED NECROSIS

Cell necrosis is distinct from cell apoptosis with swelling and membrane rupture, resulting in the loss of membrane integrity and cytoplasmic leakage (Yuan & Kroemer, 2010). Recent studies showed that programmed necrosis is an alternative route to cell death that is distinct from apoptosis in the immune system (Galluzzi et al., 2012; Han, Zhong, & Zhang, 2011; Kaiser et al., 2011; Mocarski, Upton, & Kaiser, 2011).

In the TNFR1 pathway, active caspase-8 cleaves and inactivates RIP1 (Chan et al., 2003; Lin, Devin, Rodriguez, & Liu, 1999) and RIP3 (Feng et al., 2007). When caspases are inhibited by pharmacological inhibitors or under certain physiological conditions such as viral infections, RIP1 and RIP3 form the necrosome to initiate programmed necrosis or necroptosis (Cho et al., 2009; He et al., 2009; Zhang et al., 2009). The RIP1/RIP3 complex is the core of the necrosome, which also contains other components, such as mixed lineage kinase domain like protein (MLKL).

RIP1 and RIP3 are important in host defense against bacterial and viral infections (Robinson et al., 2012; Upton, Kaiser, & Mocarski, 2010, 2012). Dysregulation of the pathway appears to be involved in many human diseases, such as lymphoproliferative diseases (Ch'en, Tsau, Molkentin, Komatsu, & Hedrick, 2011; Kaiser et al., 2011), atherosclerosis development (Lin et al., 2013), Crohn's disease (Welz et al., 2011), acute liver injury (Liedtke et al., 2011), ischemic brain injury (Degterev et al., 2005; Northington et al., 2011), myocardial ischemia–reperfusion injury (Oerlemans et al., 2012), and skin inflammation (Bonnet et al., 2011). In addition, RIP3 is responsible for the embryonic lethality of caspase-$8^{-/-}$ mice as the caspase-$8^{-/-}$ /$RIP3^{-/-}$ double knockout mice are viable (Kaiser et al., 2011). Similarly, RIP1 deficiency can rescue the lethality of $FADD^{-/-}$ mice (Zhang et al., 2011), suggesting that necroptosis plays key roles in cell development.

### 4.1. RIP1/RIP3 forms a functional amyloid signaling complex

Both RIP1 and RIP3 share a KD and a RIP homotypic interaction motif (RHIM), whereas RIP1 has one more DD at its C-terminal end (Fig. 5.5A). RIP1 and RIP3 form a necrotic signaling complex via the RHIM (Stanger, Leder, Lee, Kim, & Seed, 1995; Sun et al., 1999; Sun, Yin, Starovasnik, Fairbrother, & Dixit, 2002). The RHIM contains a high conserved (I/V/L)Q(I/V/L)G motif. Recombinant RIP1/RIP3-RHIM and RIP1/RIP3 FL complexes eluted from around the void position in gel filtration chromatography, much larger than the expected molecular weight of a heterodimer (Li et al., 2012). The complexes were identified as filamentous structures under electron microscopy (EM; Fig. 5.5B). Moreover, the RIP1/RIP3-FL and endogenous RIP1/RIP3-FL complexes purified with anti-RIP1 antibody from

HT-29 cells showed the similar filamentous core structures upon limited proteolysis to remove flanking domains and proteins.

Amyloids are fibrous protein aggregates composed of cross-β cores (Chiti & Dobson, 2006). The RIP1/RIP3 complex showed classical characteristics of β-amyloid fibrils with specific binding to amyloid-interacting dyes (Thioflavin T and Congo Red) and in Fourier transform infrared spectra (Li et al., 2012). In addition, the X-ray fiber diffraction clearly showed equatorial and meridional reflections at 9.4 and 4.7 Å resolutions, respectively, which correspond to inter- and intra-β-sheet spacings in cross-β amyloid structures (Fig. 5.5C). Additionally, in RIP3$^{-/-}$ cells reconstituted with RIP3-mCherry, necrosis induction resulted in formation of Thioflavin T (ThT)-positive clusters (Fig. 5.5D), demonstrating the physiological relevance of the RIP1/RIP3 amyloid structure (Li et al., 2012).

The recombinant and endogenous RIP1/RIP3 complex is ultrastable, consistent with the generally recognized stability of amyloidal structures (Balbirnie, Grothe, & Eisenberg, 2001). Mutagenesis in the RHIM domain and ThT staining experiments showed the core regions of RHIMs are crucial for cluster formation, kinase activity, and programmed necrosis (Li et al., 2012). In addition, both the RHIM domains and kinase activity of RIP1/RIP3 are required for TNF-induced programmed necrosis (Cho et al., 2009; Li et al., 2012). Amyloid-β amyloidogenesis occurs via a nucleated polymerization mechanism (Eisenberg & Jucker, 2012). Similarly, we propose that RIP1 and RIP3 follow a feed-forward nucleation model in which RIP1 and RIP3 kinase activation and the RIP1/RIP3 amyloid scaffold formation are mutually reinforcing (Fig. 5.5E). The amyloid scaffold may function as a crucial platform for recruiting other components, such as MLKL, and trigger the downstream execution mechanisms of necroptosis (Li et al., 2012).

### 4.2. New components of TNFR-induced cell necrosis

MLKL was recently identified as the downstream substrate of RIP3 (Sun et al., 2012; Zhao et al., 2012). Phosphorylated RIP3 can interact with MLKL and phosphorylate MLKL at sites Thr357 and Ser358. The phosphorylation on the two amino acid residues is necessary but not sufficient for necroptosis. Knockdown of MLKL can abolish TNF-induced cell necrosis. The phosphorylated MLKL may induce downstream JNK activation and reactive oxygen species (ROS) generation, eventually triggering cell death.

Another new component of the necrosome, called PGAM5 (phosphoglycerate mutase/protein phosphatase), was identified by coimmunoprecipitation withRIP3inHela cells (Wang, Jiang, Chen, Du, & Wang, 2012). Knockdown of either types of PGAM5 (long- or short-form variant) led to attenuation in TNF-induced necrosis as well as in generation of ROS and calcium ionophore in Hela cells. In contrast, knocking down RIP3 and MLKL only resulted in blockage of necrosis. The fact that PGAM5 can dephosphorylate mitochondrial fission factor Drp1 and activate its GTPase activity shows PGAM5 functions in multiple necrosis pathways (Wang et al., 2012). In addition, PGAM5L is required for NLRP3 inflammasome activation besides necrotic death (Kang, Yang, Toth, Kovalenko, & Wallach, 2013).

Interestingly, recent studies found that programmed necrosis has relationships with the NAD-dependent deacetylase SIRT2 (Narayan et al., 2012). SIRT2 binds constitutively to the C-terminal RHIM domain of RIP3. However, it is not clear whether the RHIM domain is sufficient for the interaction between the two proteins. Deletion or siRNA knockdown of SIRT2 can block the formation of the RIP1/RIP3 complex in mice. SIRT2 regulates RIP1 acetylation via deacetylation at Lys530 of RIP1, which promotes RIP1/RIP3 complex formation in TNF-induced necrosis. When SIRT2 is inhibited by a specific pharmacological

inhibitor AGK2, ischemic injury in the heart and the brain is reduced in mice (Narayan et al., 2012).

## 5. CONCLUSIONS

Members of the TNF receptor superfamily induce a diverse array of cell fates, including NF-κB activation, apoptosis, and programmed necrosis. Structural studies on the intracellular signaling complexes in these pathways have revealed that higher-order signaling machines are the central entities responsible for transmission of receptor activation information to cellular processes. Because many protein families are shared between the TNF receptor signaling pathway and other aspects of the innate and adaptive immunity system, we propose that higher-order supramolecular assemblies represent a conserved central theme in host defense. These higher-order signaling machines implicate new molecular mechanisms in proximity-driven enzyme activation, threshold behavior, and time delay of activation (Wu, 2013) that may be crucial for mounting an immune response only when the danger is sufficient and persistent to avoid accidental tissue injury associated with inflammation. Future structural and functional studies of these multiprotein assemblies may unveil the molecular basis for oligomerization-induced allosteric changes in enzyme activation in these pathways and provide new and promising avenues for therapeutic intervention.

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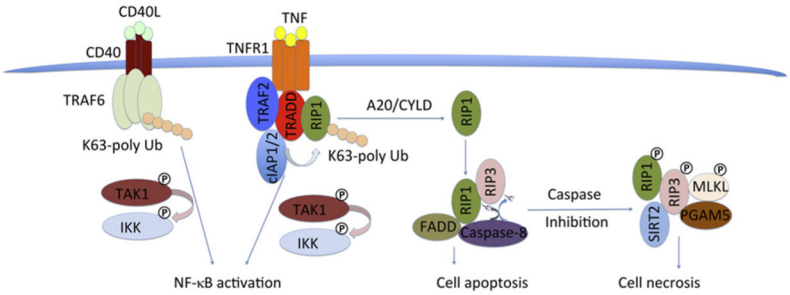


**Figure 5.1.**
Overview of signaling pathways in the TNF receptor superfamily with TNFR1 and CD40 as prototypes.

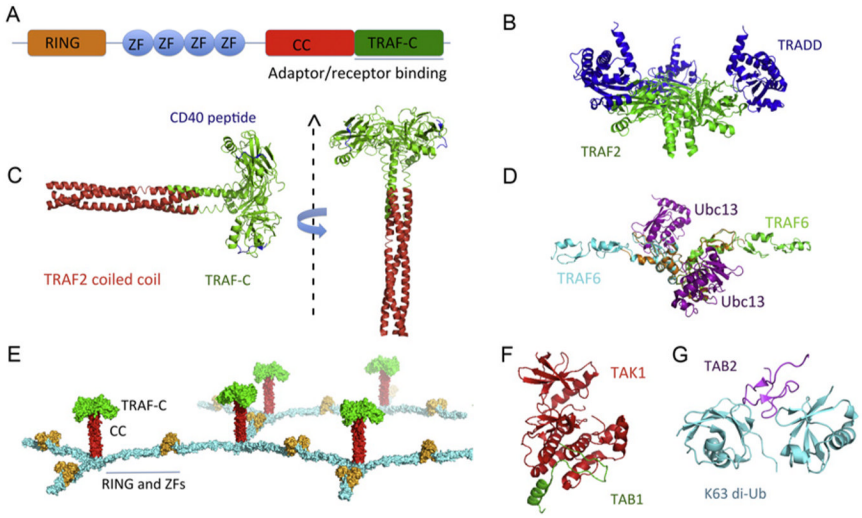


**Figure 5.2.**

TRAFs and TAK1 complex structures. (A) Domain organizations of TRAF2 and TRAF6. ZF, zinc finger; CC, coiled coil. (B) Structure of the trimeric TRADD–TRAF2 complex (PDB: 1F3V). (C) Composite C-terminal region structure of TRAF2 based on the structure of TRAF2 CC in complex with cIAP2 BIR1 and TRAF2 CC+TRAF-C in complex with the CD40 peptide, shown in two orientations (PDB: 1CZZ and 3M0A). (D) Ribbon diagram of the TRAF6 RING/ZFs/Ubc13 complex, modeled by superposition of the TRAF6 RING/ZF1/Ubc13 complex with the structure of TRAF6 RING/ZF1–3 (PDB: 3HCS and 3HCT). (E) Model of a 2-dimensional TRAF lattice assembly through trimerization of TRAF domain and dimerization of the N-terminal RING/ZF domains. (F) Structure of TAK1 in complex with an activating TAB1 peptide (PDB: 2EVA). (G) Structure of TAB2 with K63-linked di-Ub complex (PDB: 2WWZ).

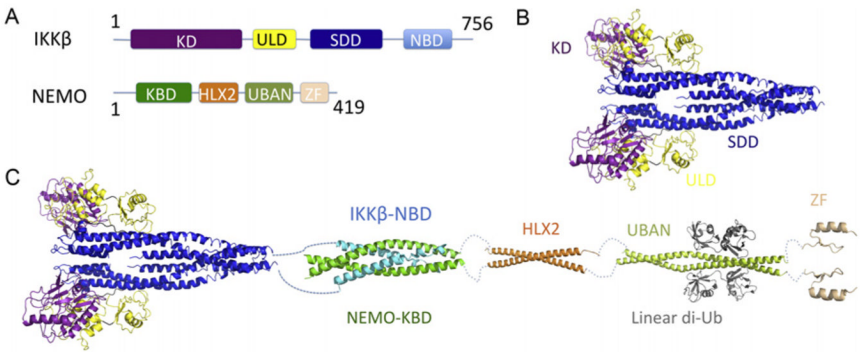


**Figure 5.3.**

The IKKβ complex. (A) Domain schematics of human IKKβ and NEMO. (B) The dimeric structure of IKKβ, consisting of the KD domain (purple), ULD domain (yellow), and SDD domain (blue). (C) Model of the full-length IKKβ–NEMO complex. The IKKβ NBD (light blue) interacts with the N-terminal kinase-binding domain (KBD) of NEMO (green). The linear representation of NEMO is based on the domain organization, with a HLX2 domain (orange), a UBAN domain (lemon), ubiquitins (gray), and a C-terminal zinc-finger (ZF) domain (light orange).

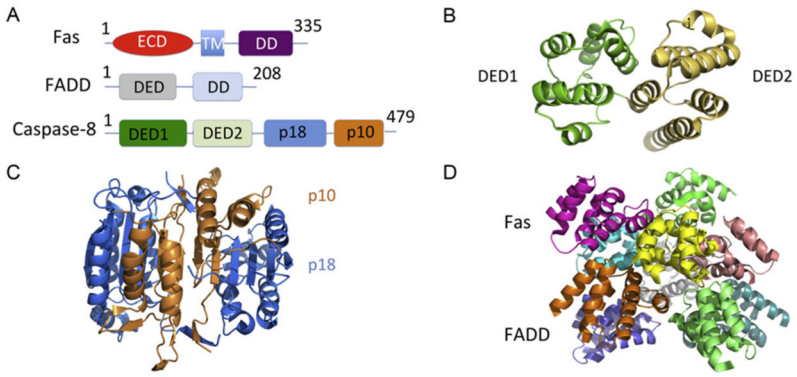


**Figure 5.4.**

Structures of the death-inducing signaling complex (DISC). (A) Domain schematics of human Fas, FADD, and procaspase-8. The Fas consists of extracellular domain (ECD, red), transmembrane domain (TM, light blue), and intracellular death domain (DD, purple). The FADD consists of DED (gray) and DD (wheat). The caspase-8 consists of N-terminal DED1 (green), DED2 (lime), and C-terminal p18 (blue) and p10 (orange) subunits. (B) The DED1/2 domain structure of vFLIP, which is a viral caspase-8/10 inhibitory protein (PDB: 2BBR). (C) The catalytic domain structures (p18 and p10) of Caspase-8 (PDB: 2Y1L). (D) The Fas DD:FADD DD complex forms a 5:5 asymmetric structure (PDB: 3OQ9).

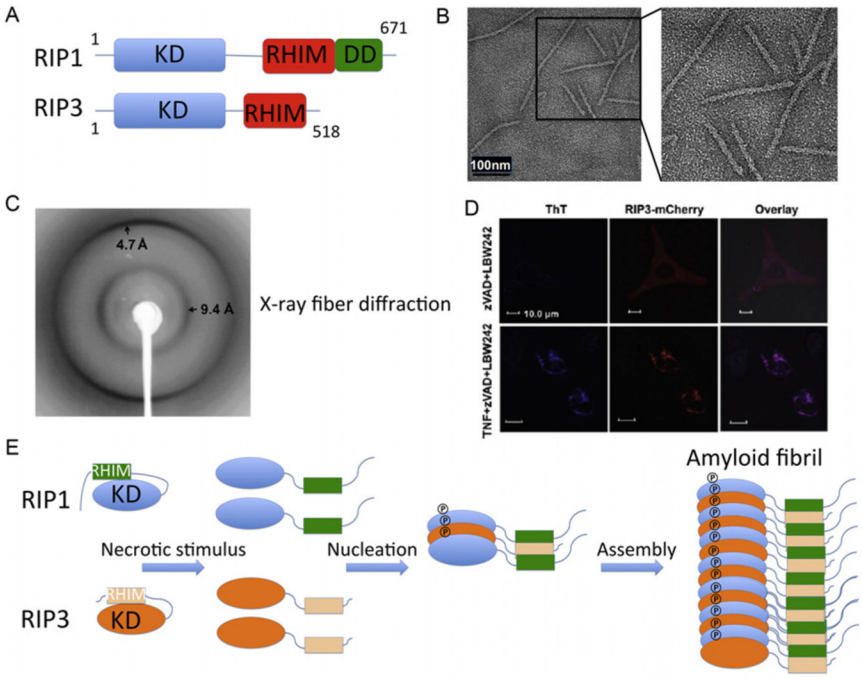


**Figure 5.5.**

RIP1/RIP3 forms a filamentous structure during TNF-induced programmed necrosis. (A) Domain schematics of human RIP1 and RIP3. (B) EM images of the RIP1/ RIP3–RHIM complex. (C) An X-ray diffraction image of partially aligned RIP1/RIP3 fibrils. (D) Colocalization of ThT with RIP3 puncta in necrotic HeLa cells. (E) A proposed model for RIP1/RIP3 fibril assembly. Phosphorylation and necrosome formation may be mutually reinforcing.