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It cuts both ways: reconciling the dual roles of caspase-8 in cell death and survival

Andrew Oberst and **Douglas R. Green***
Dept. of Immunology, St. Jude Children's Research Hospital, Memphis, TN, USA

Preface

Caspase-8 can initiate apoptosis, but it is also required for embryonic development and immune cell proliferation. While several non-apoptotic roles for caspase-8 have been proposed, recent work has indicated that the requirement for caspase-8 in development and proliferation is defined by suppression of RIPK3, a kinase that can trigger an alternative form of cell death called programmed necrosis. We will consider these recent findings, and how they can be reconciled with earlier work on the non-apoptotic roles of caspase-8.

Introduction

Caspase-8 is a cysteine protease that initiates apoptosis in response to cell surface receptors[1]. This process, referred to as "extrinsic apoptosis", is mediated by a group of receptors of the Tumor Necrosis Factor (TNF) superfamily called the death receptors (DRs). These include TNF receptor-1 (TNFR1) and the related receptor CD95 (also called Fas and APO-1)[2]. Activation of the DRs can lead to cell death, but also to proliferation and enhanced NF-kB activation as discussed below. To trigger cell death, DRs recruit an adaptor protein, Fas associated protein with a death domain (FADD), to their cytoplasmic tails; FADD then recruits caspase-8 proenzymes and activates them by inducing them to dimerize, leading to caspase activation and apoptosis (See Box 1 and Figure 1). Apoptotic caspase-8 activation is prevented by another protein, FLICE-like inhibitory protein long ($cFLIP_L$, hereafter referred to as FLIP), which is homologous to caspase-8 but lacks catalytic residues. FLIP is thereby able to form an inhibitory heterodimer with caspase-8[3] that limits apoptosis induction (Box 1). We will revisit the properties of the caspase-8-FLIP heterodimer below.

Apoptotic cell death is a means to delete superfluous or damaged cells, and as a consequence knockout of proteins involved in apoptosis often leads to defects associated with an overabundance of cells[4-8]. It is therefore surprising that knockout of caspase-8 in the mouse[9], as well as that of FADD[10, 11] or FLIP[12], leads to embryonic lethality and a failure of yolk sac vascularization and hematopoiesis first observed around developmental day E10.5. This observation led to the hypothesis that these proteins play roles in cellular processes beyond apoptosis. Conditional deletion of FADD[13, 14] or caspase-8[15] in lymphoid tissues reinforced this idea: T cells deficient for either protein—while resistant to apoptosis induced by CD95 ligation—failed to proliferate upon T-cell receptor (TCR) stimulation. The requirement of caspase-8 and FADD for cellular proliferation was extended to B cells stimulated with ligands for Toll-like receptors (TLRs) 3 and 4[14, 16, 17]. Many studies and much effort have been devoted to explaining these observations, and several non-apoptotic roles have been assigned to caspase-8, FADD, and FLIP. All three have been reported to be involved in NF-kB activation[17-20], and this requirement was proposed to explain the

*douglas.green@stjude.org.

proliferative defects of caspase-8 deficient T and B cells[17, 20]. Caspase-8 has further been implicated in cell motility[21], metastasis[20], and suppression of inflammation[22, 23], while FADD is reported to be required for normal cell cycle progression[24-26].

Several years ago, it was observed that in cultured cells in which caspase-8 or FADD are absent or inhibited, TNF treatment causes a non-apoptotic form of cell death that involves cellular swelling and rupture, features commonly associated with necrotic (rather than apoptotic) cell death[27]. Like apoptosis, this alternate form of cell death was found to be "programmed," in that it depends on activation of specific cellular enzymes: Receptor Interacting Protein Kinase-1 (RIPK1)[28] and RIPK3[29-31]. These observations led to the idea that the non-apoptotic roles of FADD and caspase-8 might involve the suppression of RIPK3-dependent programmed necrosis. This idea recently received strong genetic support, with the finding that concurrent ablation of RIPK1 or RIPK3 rescued the developmental and immune defects associated with FADD[32] or caspase-8[33, 34] deficiency. In this Opinion piece, we will summarize recent work implicating suppression of RIPK signaling as the primary non-apoptotic role of caspase-8. We then revisit and cautiously reinterpret some previously proposed non-apoptotic roles of caspase-8, FADD, and FLIP in the context of RIPK signaling.

TNF: complexes and complexities

In considering how caspase-8 might exert its suppression of RIPK signaling, we must first discuss the pleiotropic nature of signaling by the cytokine TNF. Importantly, there is currently no genetic evidence that the defects observed in caspase-8 or FADD deficient animals are due to TNF signaling. Nonetheless, TNF signaling is the best-understood framework in which to consider the interconnected pathways of NF-kB activation, apoptosis, and programmed necrosis.

The initial events following ligation of the TNF receptor have been extensively reviewed elsewhere[35, 36], and are summarized in Box 1. Importantly for the topic at hand, RIPK1 is recruited to the receptor complex (called "complex I"), and the NF-kB transcriptional program is activated. FLIP is a transcriptional target of NF-kB, and FLIP upregulation accounts for the non-apoptotic nature of the response to TNF in most cell types[37]; if FLIP upregulation is blocked, TNF signaling becomes potently pro-apoptotic[37]. As we will discuss below, FLIP is also implicated in the suppression of RIPK3-dependent signaling by caspase-8, and therefore represents a functional link between NF-kB signaling and the prevention of both apoptosis and RIPK3-dependent necrosis. (Fig. 2)

Following the formation of complex I, RIPK1 translocates to a second cytosolic complex (complex II)[38]. This translocation depends on the deubiquitination of RIPK1 by the deubiquitinase CYLD, and complex II was initially described as a site of apoptotic caspase-8 activation[38, 39]. However, when it became clear that RIPK1 also activates RIPK3 to drive programmed necrosis, and that this signaling could be blocked by caspase-8, complex II was re-envisioned as a key site of interaction between the RIPK1-RIPK3 pathway and FADD, caspase-8, and FLIP[29-31] (Fig. 2).

Two recent reports present compelling evidence that a RIPK1-containing complex capable of recruiting RIPK3, FADD, caspase-8 and FLIP may form in the cytosol independently of receptor activation[40, 41] in response to multiple cellular signals and stresses. (Box 3) These studies indicate that this complex represents a signaling "module" that is able to activate either apoptosis (via caspase-8) or programmed necrosis (via RIPK3), and can be recruited to multiple signaling platforms including TNFR or the TLR adapter TRIF. These findings suggest that the balance between caspase-8, FLIP, and RIPK signaling can determine cell

fate in response to many different types of cellular signaling and stress, a concept to which we will return.

RIPK3 & Caspase-8: Genetic evidence

RIPK3 is highly expressed in adult lymphoid tissue, and the first strong evidence that suppression of programmed necrosis might define the non-apoptotic role of caspase-8 was obtained in T cells. Initial reports held that caspase-8 deficient T-cells failed to accumulate due to proliferative defects; however, subsequent work showed that these cells proliferated normally, but did not accumulate due to increased rates of cell death[42]. Crucially, this cell death was found to be non-apoptotic, and proliferation could be restored *in vitro* by administration of the RIPK1 inhibitor Nec1[42].

Subsequent findings showed that concurrent ablation of caspase-8 and RIPK3 completely rescues developmental defects associated with caspase-8 deficiency[33, 34] – mice lacking caspase-8 and RIPK3 are born at expected Medelian frequencies and display no overt phenotype. As expected, these animals are resistant to the lethal effects of CD95 ligation *in vivo*. Furthermore, their T cells proliferate and subsequently contract normally when activated *in vivo* by a bacterial superantigen[33]. As they age, caspase-8:RIPK3 double knockout animals display a lymphoaccumulative disorder similar to that observed in mice carrying mutations that inactivate CD95 or its ligand, characterized by accumulation of an aberrant population of $CD3^+$ $B220^+$ $CD4^-$ $CD8^-$ lymphoid cells[33, 34]. Further work showed that T-cell specific deletion of caspase-8 on a RIPK3-deficient genetic background recapitulates this disorder, indicating that this cell population arises from the T-cell lineage[43]. However, the selective events that yield this population remain to be elucidated.

Animals deficient for FADD display developmental and immune-cell defects similar to those observed in caspase-8 knockouts[11]. Initial work using an inducible Cre recombinase showed that deletion of FADD in the bone marrow leads to a dramatic decrease in hematopoietic precursor cells, as well as an impaired ability to generate both lymphoid and myeloid cells[44]. Subsequent work found that concurrent ablation of RIPK1 prevents the early embryonic lethality observed in FADD deficient animals[32]. Knockout of RIPK1 alone causes perinatal lethality, and the FADD-RIPK1 double knockout animal also die shortly after birth, precluding further analysis of FADD-deficient adults.

FLIPping the survival switch

Any discussion of the non-apoptotic role of caspase-8 must address the question of how the protease is activated in the non-apoptotic context. Given that caspase-8 is a protease whose activation leads to apoptotic cell death, how can its proteolitic activity suppress RIPK3 signaling without causing apoptosis?

Intriguingly, while FLIP inhibits apoptotic activation of caspase-8 by forming heterodimers with the caspase-8 proenzyme (Box 1, Figure 1), the resulting caspase-8-FLIP heterodimers possess catalytic activity[45-47].*In vitro* and structural studies found that caspase-8-FLIP heterodimers form more readily than caspase-8 homodimers, because FLIP has greater affinity for the caspase-8 proenzyme than does the proenzyme itself [45]. This property allows FLIP to activate caspase-8 even in the absence of the interdomain autocleavage events that are necessary for stabilization and activity of the caspase-8 homodimer[45, 48]. This is important because of the finding that a mouse expressing only a non-cleavable version of caspase-8 displays normal development and immune cell activation, but that cells and tissues from this mouse are resistant to apoptosis[49]. Thus, non-cleavable caspase-8—which can be activated by heterodimerization with FLIP, but not by homodimerization—can carry out the non-apoptotic functions of caspase-8. Reconstitution of caspase-8-deficient MEF

cells with wild type or non-cleavable caspase-8 confirmed that non-cleavable caspase-8 is able to suppress RIPK3-dependent necrosis, but that it requires the presence of FLIP to do so[33]. (Fig. 2)

This work indicates that caspase-8 has distinct moieties with different functions: the caspase-8 homodimer causes apoptosis, and the catalytically active caspase-8-FLIP heterodimer, whose activity suppresses RIPK3 signaling and mediates the non-apoptotic role of caspase-8[33] (Figure 2). Importantly, when apoptosis is blocked downstream of caspase-8 activation, it was found that FLIP is nonetheless required to prevent RIPK3-dependent necrosis; that is, activation of the caspase-8 homodimer is not sufficient to prevent RIPK3-dependent necrosis[33]. Because FLIP is a transcriptional target of NF-kB, this work identifies FLIP upregulation as a key mechanism by which NF-kB signaling prevents both apoptosis and RIPK3 activation (Fig. 2). However, the mechanism by which the caspase-8-FLIP complex suppresses RIPK signaling is currently unknown. Both RIPK1[50] and RIPK3[51] have been described to be caspase-8 substrates, and a recent study showed that a cleavage mutant of RIPK1 could trigger necrosis even in the presence of caspase-8 activity[52]. Another recent study identified the deubiquitinase CYLD as a key substrate of caspase-8 in the prevention of RIPK-dependent necrosis[53], though the complex in which the cleavage occurs *in vivo* remains to be elucidated. (Box 3)

The above treatment of FLIP signaling has considered only the long isoform of FLIP; however, a short version of FLIP ($FLIP_S$), composed only of the prodomain, also exists[54]. $FLIP_S$ can inhibit caspase-8 activation in a dominant-negative fashion by competing for binding to FADD, but it does not possess the ability to activate caspase-8 by heterodimerization. Consistent with the model proposed above, $FLIP_S$ is not able to suppress RIPK activation[55]. This distinction is important because many viruses encode anti-apoptotic proteins homologous to $FLIP_S$, and programmed necrosis has been implicated in anti-viral responses[29, 56].

Caspase-8: roles beyond apoptosis

The data outlined above provide strong genetic support for the non-apoptotic functions of caspase-8 and FADD depending on the suppression of the RIPK1-RIPK3 signaling pathway[32-34]. In light of these findings, it is worthwhile to consider some non-apoptotic roles previously assigned to caspase-8 and FADD.

NF-kB signaling

Several reports have sought to explain the defects observed in caspase-8, FADD or FLIP deficient embryos and immune cells by implicating these proteins in the induction of NF-kB signaling (Box 2). The catalytic activity of caspase-8 was shown to be required for NF-kB activation in antigen-stimulated T cells, and caspase-8 was also found to associate with the CARMA-Bcl10-MALT-1 complex, which is required for NF-kB activation following antigen receptor ligation in T- and B-cells[57]. Caspase-8 was further shown to interact directly with TRAF family members, which promoted translocation of caspase-8 into lipid rafts[58], and additional work implicated lipid rafts as the site of formation of the caspase-8-FLIP complex and its association with several proteins involved in NF-kB activation[59]. Indeed, FLIP has also been implicated in NF-kB activation, with a number of studies indicating that one or more of the FLIP cleavage fragments generated by association with caspase-8 are involved in NF-kB activation[19, 60]. Some caution must be used in interpreting these studies, as they present work from both murine and human systems. Unlike mice, humans have the close caspase-8 homologue caspase-10[61,62], which displays regulatory and functional properties distinct from caspase-8. How the recent discoveries in murine systems will translate to human models remains an open question.

Nonetheless, the idea that caspase-8 plays a role in NF-kB activation has met with some recent setbacks. The timing and tissues involved in embryonic lethality of caspase-8-deficient mice are distinct from those observed in NF-kB-related knockouts—the latter die around E13-14 due to liver defects[63, 64]. Furthermore, caspase-8-RIPK3 double-knockout T-cells proliferate normally[33], without any of the defects observed in T cells lacking NF-kB components[63], Bcl10, CARMA-1 or MALT-1[65-67]. Indeed a careful analysis of T cells indicated that caspase-8-deficient cells have normal NF-kB activation, and that the defects associated with caspase-8-deficeincy (initial proliferation followed by necrotic death) are distinct from those observed in cells lacking NF-kB signaling (lack of proliferation)[42]. These findings similarly raise questions about the proposed role of caspase-8-cleaved FLIP in NF-kB activation, as caspase-8 deficiency would presumably abolish this protein species. Indeed, careful analysis of FLIP-deficient T[68] and B[69] cells revealed that, while both cell types displayed an increased susceptibility to cell death, NF-kB signaling remained intact.

Beyond T-cell activation, both caspase-8 and FADD have also been reported to be required for NF-kB activation and proliferation in B cells, in response to the innate pattern recognition receptors TLR3 and TLR4[17, 70]. In considering the role of FADD and caspase-8 in this context, it is interesting to note that both TLR-3 and TLR-4 signal through the adapter protein TRIF. This protein contains a Rip Homotypic Interaction Motif (RHIM) domain, and is able to recruit RIPK1 and RIPK3[71]; it has also been reported to trigger FADD- and caspase-8 dependent apoptosis[72]. A recent report indicated that a RIPK1-containing complex capable of triggering both apoptosis and RIPK3-dependent necrosis is recruited to TRIF following TLR-3 ligation[40]. This report also showed the presence of FLIP in this complex, and a requirement for this molecule in limiting caspase-8 activation and suppressing RIPK3-dependent necrosis. Together, this work strongly implies that the requirement for FADD, caspase-8, and FLIP in TLR signaling is defined by suppression of RIPK3.

Caspase-8 and inflammation

Several reports have implicated caspase-8 in the suppression of inflammation. It was initially observed that, in mice in which caspase-8 was conditionally deleted in hepatocytes, partial hepatectomy led to a blunted proliferative response followed by chronic inflammation of the liver[73]. Subsequently, it was found that conditional deletion of caspase-8 in basal keratinocytes led to severe and fatal inflammatory skin disease, which could be partially ameliorated by knockout of TNF. Caspase-8 deficient epidermal cells displayed increased inflammatory signaling in response to transfected DNA, which raised the possibility that caspase-8 could function to suppress an inflammatory pathway downstream of an innate nucleotide sensor[23]. Subsequent work using sendai virus infection as a model system showed that caspase-8 suppresses activation of the pro-inflammatory transcription factor IRF-3 by the cytosolic RNA sensor RIG-I[22]. Significantly, this suppression was shown to be mediated by cleavage of RIPK1, which is recruited to a complex containing RIG-I and its adapter, MAVS (Box 3).

Here again, the phenotype of the caspase-8:RIPK3 knockout must be taken into account. Unlike the severe epidermal inflammation observed in skin-specific knockout of caspase-8, skin from the caspase-8-RIPK3 double knockout animal was entirely normal[33]. This implies that whatever defect provokes inflammation in the absence of caspase-8 is corrected by removal of RIPK3. Intriguingly, epidermal ablation of NF-kB signaling also causes severe inflammation[74]. FLIP connects NF-kB signaling to suppression of the RIPK1-RIPK3 pathway by caspase-8; the possibility therefore emerges that commonalities between epithelial deletion of caspase-8 and NF-kB signaling could be due to disregulation of RIPK1-RIPK3 signaling.

Recent findings from another epithelial model—the gut—also support these ideas[75]. This study found that FADD deletion in the gut led to inflammatory bowel disease, an effect that was rescued by concurrent ablation of RIPK3. Importantly, the inflammatory phenotype associated with FADD deletion was also ameliorated by deletion of TNF, the innate immune adapter Myd88, or elimination of gut microbiota. Similarly to the skin model discussed above, this finding presents the specter of unrestrained RIPK signaling leading to a multi-faceted, self-reinforcing inflammatory response, with TNF signaling and gut barrier breakdown both contributing. NF-kB deletion in this context would be expected to sensitize cells not only to RIPK3-dependent effects, but also to apoptosis—an outcome that caspase-8 or FADD deletion obviously prevents. Indeed, it was found that deletion of CYLD—which is required for RIPK activation downstream of TNF—prevented inflammation caused by intestinal deletion of FADD, but not of NF-kB signaling[75]. This implies that both apoptosis and RIPK-dependent necrosis contribute to the inflammatory effects of NF-kB deletion; analyzing these effects in a tissue other than the gut, where any cell death can cause barrier breakdown and thus severe inflammation, will be informative in delineating the relative inflammatory contributions of apoptotic and necrotic cell death.

Concluding Remarks

Recent work provides strong genetic evidence for the idea that suppression of RIPK1-RIPK3 signaling defines the non-apoptotic roles of FADD and caspase-8. Because caspase-8, FADD and FLIP are able to suppress RIPK1-RIPK3-mediated programmed necrosis in cultured cells, it is tempting to conclude that prevention of programmed necrosis defines the non-apoptotic role of these proteins. However, because the pathways of programmed necrosis are so poorly understood, all that can be said definitively is that the death of caspase-8-deficient embryos is RIPK3-dependent; what cellular processes are mediated by RIPK3 in this context, and what signaling events trigger them, remain unknown. The known roles or RIPK1 and RIPK3 in TNF and innate immune signaling make it tempting to reinterpret the proposed non-apoptotic roles for caspase-8, FADD and FLIP in these pathways, as we have done. However, we have not been exhaustive. FADD has been implicated in cell cycle progression[24-26], while caspase-8 has been shown to promote cell motility and affect tumor metastasis[21]. It is possible that these functions are independent of RIPK1-RIPK3 signaling, but are simply not required for normal development and survival and therefore do not manifest in double-knockout mice. However, the intriguing possibility exists that these effects of FADD and caspase-8 are due to modulation of unknown effects of RIPK1-RIPK3 signaling. Rigorous reevaluation of possible roles for RIPK signaling in these contexts is needed, and until the downstream effects of the RIPK pathway are understood, we cannot rule out their involvement.

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Box 1

The Mechanism of caspase-8 activation

The caspases are present in inactive forms in most cells types. Caspases are synthesized as proenzymes composed of a central large subunit (~20 kilodaltons) and a C-terminal small subunit (~10 kilodaltons); in addition to these domains, the initiator caspases (such as caspases-8, 2 and 9, the enzymes that initiate apoptotic signaling) also possess an N-terminal prodomain that mediates protein-protein interactions[76]. Initiator caspase proenzymes remain monomeric until they are recruited to large activation platforms via interactions with their prodomains. These large molecular weight platforms include the DR- and RIPK1-associated complexes described herein for caspase-8, and the cytochrome-c/APAF-1 complex called the “apoptosome” for caspase-9. Once recruited to these platforms, caspase proenzymes are induced to dimerize, and this dimerization leads to enzyme activation[77]. In the case of caspase-8, this dimerization is followed by interdomain cleavage events, first between the large and small subunits and subsequently between the large subunit and the prodomain. These cleavage events stabilize the caspase-8 homodimer and remove the prodomain, leading to formation of a fully active enzyme composed of two large and two small subunits[78]. Importantly for the current topic, a point mutation that prevents the stabilizing cleavage of caspase-8 between the large and small subunits prevents activation of caspase-8 by prodomain-driven homodimerization[79, 80]. However, this non-cleavable mutant is still able to be activated by heterodimerization with the caspase-8-like protein FLIP[45,48, 81]. This caspase-8-FLIP heterodimer is implicated in carrying out the suppression of RIPK3 signaling that defines the non-apoptotic role of caspase-8. However, how this suppression takes place, and how the caspase-8-FLIP complex is prevented from inducing apoptosis remains unclear. Artificial formation of caspase-8-FLIP heterodimers independent of DR ligation using an inducible-dimerization system readily triggers apoptosis[48], indicating that catalytic differences between the homo- and heterodimer are not sufficient to explain their distinct functions. It is likely that receptor-mediated signaling exerts additional controls on the caspase-8-FLIP complex, such as restricted localization or rapid degradation, that further limit its cleavage of apoptotic substrates.

Box 2

Activation of NF-kB by TNF receptor

When TNF binds to its trimeric receptor, it induces a conformational change that results in the recruitment of RIPK1 and the TNF receptor associated protein with a death domain (TRADD) to the cytoplasmic DD-containing tail of TNFR1[38] (1). These proteins subsequently recruit members of the TNFR-associated factor (TRAF) family, as well as cellular inhibitor of apoptosis (cIAP)-1 and -2[35]. The cIAPs are E3 ubiquitin ligases which catalyze addition of ubiquitin moieties via non-degradative K63 linkages to RIPK1 and TRAF-2, as well as to the cIAPs themselves (2, black arrows); these modifications in turn allow recruitment of the linear ubiquitin chain assembly complex (LUBAC), which catalyzes addition of linear ubiquitin chains to multiple members of the complex, an event that is believed to stabilize these interactions and allow efficient, sustained signaling[82, 83](3). NF-kB essential modulator (NEMO), the core component of the IkB kinase (IKK) complex, is among the ubiquitination targets of LUBAC; this modification stably recruits NEMO and the IKK complex. The IKK complex is then phosphorylated and thereby activated by kinases TAB2 and TAK1, which associate with ubiquitin linkages attached to RIPK1[84] (4). IKK complex activation leads to phosphorylation and proteasome-mediated degradation of the inhibitory molecule IkB, which in turn allows activation and nuclear translocation of the NF-kB transcption factor complexes[35], and transcriptional upregulation of NF-kB targets, including FLIP. Following these events, ubiquitin chains are removed from RIPK1 by the deubiquitinase CYLD (5), which allows RIPK1 to translocate to the cytosol to form a second complex; this is believed to be the site of functional interaction between RIPK1, RIPK3, FADD, FLIP, and caspase-8[29, 39, 55] (Described in Fig. 2).

Box 3

The role of ubiquitination

In considering the dynamics of receptor activation, apoptosis, and programmed necrosis, it is useful to note the central role of ubiquitination in these processes. Both degradative and non-degradative ubquitination plays a central role in the control of NF-kB activation, as well as caspase-8 and RIPK1 signaling[85, 86]. Ubiquitin chains can be linked via several different lysine residues in the ubiquitin monomer, and recent reports show that RIPK1 is targeted by 4 separate types of ubiquitin linkages during receptor signaling[87]. The deubiquitinase CYLD is required remove ubiquitin chains from RIPK1, allowing formation of complex II[39], and caspase-8 may block programmed necrosis by preventing this process[53]. More recent reports indicated that degradative ubiquitination by cIAP and XIAP prevent receptor-independent formation of a RIPK1-containing complex, termed the “ripoptosome,” that is capable of triggering caspase-8-mediated apoptosis as well as RIPK3-dependent necrosis[40, 41]. Furthermore, in the context of suppression of RIPK1-mediated inflammation at the RIG-I complex by caspase-8, it was reported that K63-linked RIPK1 ubiquitination is required to render RIPK1 susceptible to caspase-8-mediated cleavage, the mechanism by which RIPK1 signaling is reported to be suppressed in this context[22]. Caspase-8 has also been reported to be modified by non-degradative ubiquitination chains, and intriguingly this modification was reported to allow activation of non-cleavable caspase-8, a mutant able to suppress RIPK signaling but not trigger apoptosis[85]. It is therefore possible that dynamic ubiqutination is at least partially responsible for controlling the activation of caspase-8 by FLIP (see Box 1). Degradative ubiquitination of the caspase-8-FLIP complex has also been reported[41], and the rapid proteosomal degradation of this complex could explain how it is prevented from triggering apoptosis. The study of the role of these and other posttranslational modifications in the dynamics of NF-kB activation, apoptosis and programmed necrosis will be crucial to understanding these interrelated processes.

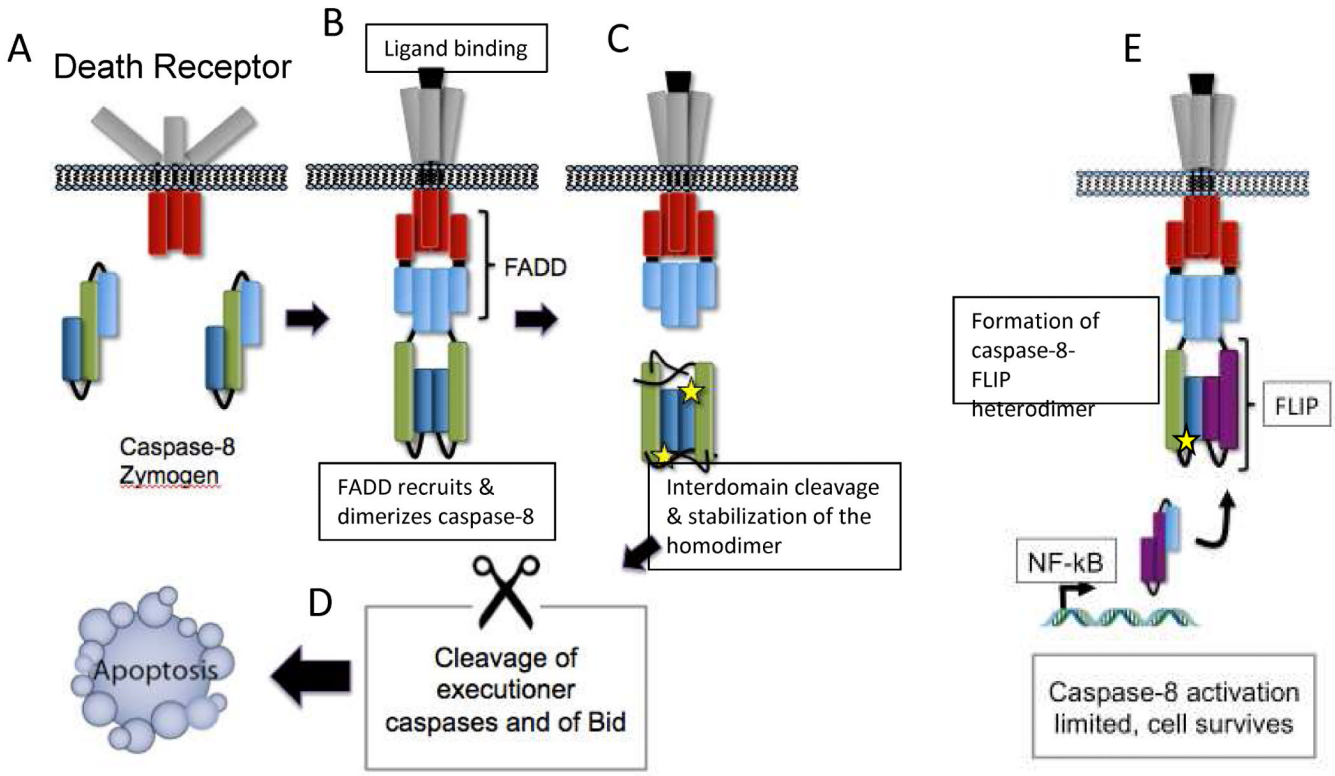

Fig. 1.

Activation of caspase-8 by homo- and heterodimerization.

This Figure depicts activation of caspase-8 at the receptor-associated DISC; however, similar homo- and heterodimerization events take place in the RIPK1-associated complex depicted in Fig. 2. A) Inactive caspase-8 zymogens are present in the cytosol of most healthy cells. These are composed of a prodomain (light blue), and one large and one small subunit (green and dark blue, respectively). B) Ligation of cell surface receptors such as CD95 leads to recruitment of the adapter FADD, which in turn recruits monomeric caspase-8 zymogens present in the cytosol via interactions with the caspase-8 prodomain. C) When FLIP levels are low, this recruitment leads to homodimerization, which is followed by cleavage of the interdomain linker regions. These cleavage events stabilize the homodimer and allow formation of the proteolytic active sites, symbolized by stars[79]. D) The fully active caspase-8 homodimer can then transduce the pro-apoptotic signal by activating downstream caspases, or by cleaving and activating the Bcl-2 family member Bid. E) When FLIP levels are high (e.g. following NF-kB activation by the TNF-R1-associated complex I; see Box 2), caspase-8 preferentially recruits and homodimerizes with FLIP[45]. The caspase-8-FLIP heterodimeric complex is catalytically active, and importantly FLIP can activate caspase-8 in the absence of interdomain cleavage events[48]. The activity of the caspase-8-FLIP complex does not trigger apoptosis, and is responsible for the suppression of RIPK1-RIPK3 signaling[33]. However, the key substrate(s) of this complex, as well as how FLIP limits caspase-8 activation *in vivo*, remain to be elucidated.

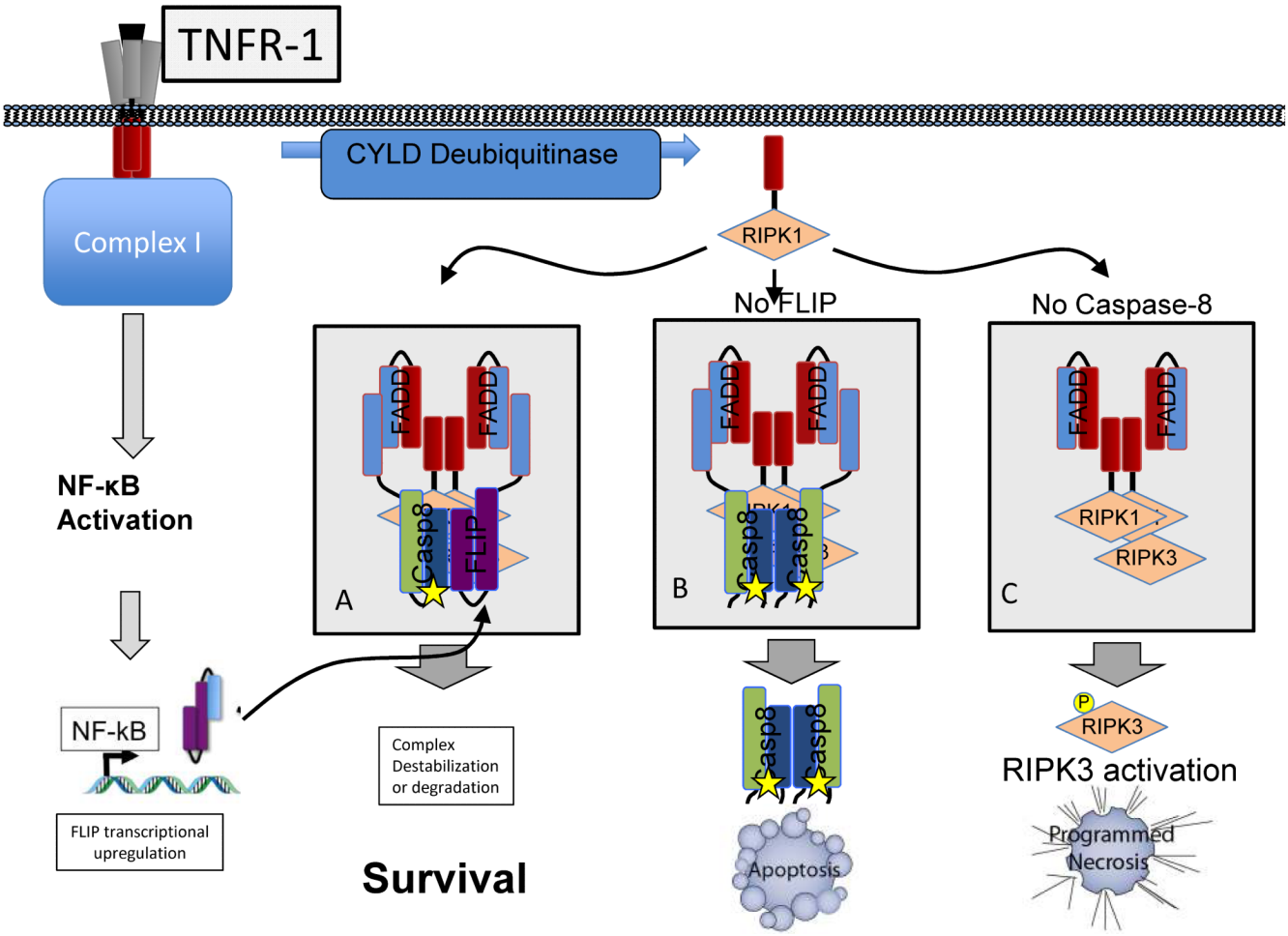

Fig. 2.

Recruitment of caspase-8 and FLIP to a RIPK1-containing complex determines cell fate. This Figure depicts the formation of the RIPK1 containing, RIPK3 activating complex II following TNFR1 ligation; however, recent evidence indicates that a similar complex can be induced by TLR-3 or -4 ligation or genotoxic stress[40, 41]. TNFR1 ligation initially triggers NF-kB activation and transcriptional upregulation of FLIP (see Box 2). RIPK1 is then deubiquitinated and translocates to the cytosol, where it can recruit FADD, caspase-8, and/or FLIP in a manner analogous to that depicted in Fig. 1. RIPK1 can also recruit and activate RIPK3 in this complex, a process that is controlled by FADD, caspase-8 and FLIP. A) When both caspase-8 and FLIP are present, these proteins are recruited to the RIPK1-containing complex. The caspase-8-FLIP heterodimer limits RIPK1-RIPK3 signaling, but does not trigger apoptosis. Importantly, the mechanism by which suppression of RIPK1-RIPK3 signaling by the caspase-8-FLIP complex is carried out remains controversial[52, 53]. B) When FLIP levels are low (for example, if NF-kB signaling is prevented), caspase-8 activation is unchecked, leading to apoptosis[40, 41]. FLIP is required not only to limit caspase-8 activation, but also to for suppression of RIPK signaling[33], so reduced FLIP levels can also sensitize cells to RIPK3-dependnent necrosis if apoptosis is prevented. C) When caspase-8 (or FADD) is absent, apoptotic activation of caspase-8 is prevented, but RIPK signaling proceeds unchecked. The result is sensitization to RIPK3-dependent programmed necrosis.