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Authors
Salvesen, Guy S
Walsh, Craig M

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Functions of Caspase 8: the Identified and the Mysterious

Guy S. Salvesen and
Program in Cell Death and Survival Networks, Sanford-Burnham Medical Research Institute, La Jolla, CA 92037, USA

Craig M. Walsh
Department of Molecular Biology and Biochemistry, Institute for Immunology, University of California, Irvine, CA 92697, USA

Guy S. Salvesen: gsalvesen@sanfordburnham.org; Craig M. Walsh: cwalsh@uci.edu

Abstract

Initially discovered as an initiator protease in apoptosis mediated by death receptors, caspase-8 is now known to have an apparently confounding opposing effect in securing cell survival. It is required to allow mouse embryo survival, and the survival of hematopoietic cells during their development and activation. Classic models in which caspase-8 is depleted or inhibited frequently result in inhibition of apoptosis, and conversion to death through a necrotic pathway. This bewildering switch is now known to be driven by activation of a pathway dependent on protein kinases of the RIP family, which engage a pathway known as necroptosis. If caspase-8 does not control this pathway, necrotic death results. The pro-apoptotic and pro-survival functions of caspase-8 are regulated by a specific interaction with the pseudo-caspase cFLIP, and it is thought that the heterocomplex between these two partners alters the substrate specificity of caspase-8 in favor of inactivating components of the RIP kinase pathway. The description of how caspase-8 and cFLIP coordinate the switch between apoptosis and survival is just beginning. The mechanism is not known, the differential targets are not known, and the reason of why an apoptotic initiator has been co-opted as a critical survival factor is only guessed at. Elucidating these unknowns will be important in understanding mechanisms and possible therapeutic targets in autoimmune, inflammatory, and metastatic diseases.

Keywords

apoptosis; necrosis; necroptosis; autophagy

1. Introduction: a little history

1.1 Traditional role of caspase 8 in extrinsic apoptosis

The great genomics rush of the mid 1990s was a watershed event in the apoptosis field, where new members of the caspases and the Bcl-2 family were discovered on what seemed like a monthly schedule. In 1992, apoptosis was gaining interest following the revelations of the existence of a genetically-programmed pathway in *C. elegans* [1], but nobody had much of an idea of how the mammalian pathway was regulated. By 1998 pretty much all of the currently known protein components that participate in apoptosis had been defined in humans and laboratory mice [2, 3]. Spurring these advances was the discovery that caspases were comparatively easy to express in *E. coli* in active forms [4, 5], allowing for relatively straightforward characterization of the properties and fundamental distinguishing characteristics of these proteases, at least *in vitro* [6–8]. Exemplifying this trend was caspase 8 (casp8).

It was known that death ligands such as FasL and TNF (Tumor Necrosis Factor) transmit information from outside a cell to the cytosol by engaging their cognate receptors, via cytosolic adaptor molecules [9], and it was the discovery of casp8 through EST homology analysis [10] and interactive cloning [11] that paved the way to reveal the first proteolytic signal in the initiation of the extrinsic pathway of apoptosis.

1.2 New role in protection against RIPK-dependent death (necroptosis)

Given that casp8 was thought to be the primary mediator of extrinsic apoptosis (but see below for a discussion of caspase 10), it came as a surprise that deletion of the gene in mice [14] resulted in embryonic lethality with a phenotype reminiscent of degeneration rather than proliferation, an observation brought home by the discovery of a casp8 mutation in humans that decreased immune activation of naive lymphocytes [15]. The most parsimonious explanation for these apparently counterintuitive findings was that casp8 had dual roles: one pro-death and one pro-survival.

It had long been known that engagement of the (DR) receptor TNFRI in many cell types provided a proliferative stimulus that could be converted to apoptosis by treatment with protein translation inhibitors. This was classic casp8 mediated apoptosis. But treatment with the broad-spectrum caspase inhibitor Z-VAD-FMK (benzoxycarbonyl-Val-Ala-Asp-fluoromethyl ketone) paradoxically also resulted in cell death, with kinetics sometimes faster that the apoptotic outcome [16]. TNFRI engagement triggered another death pathway, and this pathway was countered by casp8, putting flesh onto the idea of a pro-survival role. Breakthroughs in understanding the putative pro-survival role were provided by a chemical biology approach that identified RIPK1 (Receptor Interacting Protein Kinase 1) as a mediator of the second death pathway [17] – frequently called necroptosis –with final validation by intercrossing mice defective in casp8 and/or RIPK1 and RIPK3 – reviewed in [18].

2.1 Activation Mechanism

To understand the pro-apoptotic and pro-survival roles of casp8 it is important to comprehend the mechanism of activation of this protease. All caspases are obligate homodimers in their active forms. The two monomers of the active molecule are required to provide mutual interactions that stabilize the catalytic site in a productive conformation [22, 23]. This means that caspases typically have two active sites – one per monomer. Effector caspase zymogens (Fig 1) are pre-formed dimers and require proteolysis in an intra-domain linker, which effectively releases a lock on the zymogen form, to allow transition to the catalytically competent conformation. In contrast, apical caspase zymogens are monomers, and simply require dimerization to gain catalytic competency. Thus the general mechanisms of caspase zymogen activation represent a perfectly harmonized pathway – when an apical protease is activated its catalytic power is used to activate a downstream member by proteolysis. The concept is somewhat comparable with receptor tyrosine kinase signaling, where receptor clustering activates an apical kinase and its catalytic power is used to activate a downstream member by phosphorylation [24].

The fundamental concept for apical caspase activation – generation of the first protease active site - is described by the “induced proximity” model, introduced in its initial [25] and refined [26] forms. In essence, of the induced proximity model stipulates that apical caspases exist in their quiescent state as monomers that must be *dimerized* to gain activity. This is achieved on *oligomeric* signaling platforms, either the soluble “apoptosome” that activates caspase 9, or the membrane-associated DISC that activated casp8 and 10 [27]. These platforms serve to recruit monomers via homotypic recruitment domains, clustering the monomers in space and thereby enabling them to attain the dimeric, active, conformation. The concept is straightforward: clustering converts a three-dimensional diffusion process, where the K_d for dimerization is unfavorable, to a two-dimensional process that overcomes this unfavorable K_d [26, 28, 29]. Importantly, and unfortunately, this concept is sometimes mis-represented in the literature because the oligomerization of the platform (the driving force) is confused with the dimerization of the caspase (the activation process) and it is misleading to say that caspases are activated by oligomerization – it is correct to say that they are activated by dimerization within oligomeric platforms [30]. This mechanism has predictions that have been tested and validated [31, 32], with consequences for the specificity of death *versus* survival activity of casp8.

2.2 Substrate Specificity and the FLIP Switch

The homologue of caspase-8 referred to as cellular Flice Inhibitory Protein (cFLIP), which contains DEDs and therefore is also recruited to the DISC, contains inactivating mutations in the catalytic and substrate binding sites of the caspase homology domain (Fig 2). The short isoform of cFLIP ($FLIP_S$) lacks the catalytic domain and therefore can, in principle, act as a negative regulator of DISC formation. However, the long form ($FLIP_L$) contains the entire casp8 homology domains and confers polymorphic phenotypes upon cell transfection. $FLIP_L$ was concurrently identified by several groups, and data presented in these publications were controversial regarding whether $FLIP_L$ was pro- or anti-apoptotic. Some thought $FLIP_L$ to be an inhibitor of DISC formation and casp8 activation, hence the name,

and others thought it to be an independent casp8-like cell death inducer [40–44]. This controversy was settled when it was recognized that $FLIP_L$ prevented cell death upon over-expression by occupying the majority of binding sites for caspase-8 at the DISC [45], but the field has now moved ahead and it seems that FLIP has an entirely different mechanism in securing cell survival.

Heterodimerization with $FLIP_L$ activates caspases 8 and 10 [46–48] because $FLIP_L$ utilizes the same dimer interface between the catalytic domains [49]. Thus $FLIP_L$ could be considered an activator of caspases 8 and 10. Perhaps more importantly, $FLIP_L$ is actually a better activator of casp8 and 10 than are these caspases themselves, because the entropic barrier for heterodimerization is lower than for homodimerization [46, 50]. Thus one could propose, although it is currently very difficult to test, that in the presence of $FLIP_L$ the first active enzyme at the DISC may actually be the casp8 (or 10) heterodimer with $FLIP_L$. In purified systems, the heterodimer has a restricted substrate repertoire and reduced activity on the main pro-apoptotic targets downstream of caspase-8/10 activation, such as caspase-3 and Bid, as demonstrated in two independent studies utilizing *in vitro* generated caspase-8/$FLIP_L$ heterodimers [30, 50].

Extensive literature on post-translation regulation of casp8, primarily involving Tyr phosphorylation – reviewed in [33], and K63 [34] and K48 [35, 36] ubiquitylation, is available and readers are referred to these publications and additional reviews for thorough explorations of caspase post-translation modifications [37, 38]. We have not included a review of these here, since there is no evidence yet that the survival/death role of caspase-8 is impacted by such modifications, although this would be a fertile area to explore. However, we note that cFLIP isoforms are subjected to active isoform-specific ubiquitylation and degradation. Thus, $FLIP_S$ is degraded more rapidly that $FLIP_L$ [39], with the implication that the DISC-inhibitory function may persist more transiently than the casp8 and 10 activation function.

2.3 Evolution of caspases 8, 10 and cFLIP

By comparing the genome databases of caspases that have been identified in a wide variety of animals it seems that the sub-group of caspases of the extrinsic pathway, including the ancestors of caspases 8, 10 and cFLIP, was newly established in the fish lineage and later underwent duplication, variation, and deletion events in the mammalian lineage during evolution [51] – see Fig 2. If a function of $cFLIP_L$ is indeed to activate casp8, we predict this would also be true for the earlier casp8 paralogs, with the understanding that the survival function of casp8 goes back at least to fish. In this context, we note that the RIPK and MLKL components of the necroptosis pathway were already in place before the origin of bony fish, and thus predated the cFLIP and caspase-8 progenitors (Kay Hoffman, personal communication).

2.4 T/B cell caspase 8 – activation pathway

Perhaps one of the earliest clues that casp8 activity was not exclusively engaged in promoting apoptosis was uncovered through examination of lymphocytes following their antigen receptor stimulation. Numerous studies implicated death receptors (DR) of the TNF

receptor family in the regulation of lymphocyte homeostasis and tolerance [53, 54]. *Lpr* mice, bearing a splicing defect in the Fas/CD95 gene, were found to present with lymphoproliferative disease [55]. Other studies revealed that mice bearing the *Gld* mutation [56], a mutation in Fas/CD95 ligand, also developed lymphadenopathies and lymphoproliferative diseases that were highly similar to those observed in *Lpr* mice [57, 58]. These studies supported the hypothesis that DR signaling via Fas/FasL is crucial to the maintenance of lymphocyte homeostasis. It was thus surprising that mice lacking the function of FADD [59, 60], an essential signaling adaptor required for DR-induced apoptosis [61], failed to develop lymphoproliferative disease [62–64]. Subsequent investigation also revealed that loss of casp8, normally recruited to the death inducing signaling complex (DISC) via FADD following DR stimulation [10, 11, 60, 65], similarly failed to cause lymphoproliferative disease in humans and mice [15, 66]. Supporting the notion that FADD and casp8 are required for processes distinct from caspase-induced apoptosis, germline deficiencies in FADD and casp8 result in embryonic lethality at roughly E10 [14, 64, 67]. Curiously, germline deletion of the non-catalytic casp8 homologue cFLIP also results in embryonic lethality and analogous lymphocyte proliferation defects [66, 68–70].

FADD, casp8 and cFLIP all are required for T and B cell clonal expansion, and also for lymphocyte homeostasis. Loss of FADD signaling via engineered null mutation or through ectopic expression of a dominantly interfering isoform, leads to significant T cell proliferative defects [62–64]. Such mice were found to have diminished peripheral T cell populations [71], especially CD8+ cells, demonstrating an important role for this DR adaptor in CD8+ T cell homeostasis and antiviral immunity [72]. Subsequent studies have revealed a similar requirement for FADD in proliferating B cells, although in B cells the proliferative defect appears to be dependent on the stimulus [73]. While FADD-deficient B cells respond normally to B cell receptor stimulation, Toll-like receptor (TLR) stimulation results in defective clonal expansion. casp8 and cFLIP function are similarly vital to lymphocyte cell clonal expansion and homeostasis. Loss of casp8 by virtue of conditional deletion in mice results in defective T cell proliferation and homeostasis [66], primarily due to enhanced cell death [74]. casp8 activity is required in B cells for stimulus dependent mitogenesis in a manner essentially identical to that of FADD [75]. cFLIP is also required for normal T cell mitogenesis and homeostasis, although its role in these processes is more complex due to its anti-apoptotic nature [69, 70, 76, 77]. Unsurprisingly, cFLIP also required for B cell proliferation and in vivo responsiveness [78, 79]. Taken together, FADD, casp8 and cFLIP all promote non-apoptotic signaling required for lymphocyte clonal expansion, homeostasis and immune function [80].

The basis for the proliferative defects observed in lymphocytes bearing defects in DISC factors (e.g. FADD, casp8, cFLIP) was the source of much consternation; how could it be that loss of a signaling cascade required to promote apoptosis not recapitulate the phenotype observed with the absence of the ligand or receptor at the apex of the cascade? It was clear that there must be divergence of function downstream of the DR, and the most obvious place for this would be at the level of casp8 activity. Indeed, caspase activity was observed in antigenically stimulated T cells, and the pan-caspase inhibitor Z-VAD-FMK was found to

block clonal T cell expansion [81, 82]. However, the requirement for casp8 catalytic activity in T cell clonal expansion was not established until recently when it was observed that reconstitution of casp8 with wildtype, but not a catalytic mutant, restored the proliferation of casp8-deficient T cells [83]. This antigen receptor induced casp8 activity appears to be distinct from that induced by the DISC assembled around a ligated DR. DR ligation results in the catalytic activation (via induced proximity) and proteolytic cleavage of the casp8 p12 light chain [25, 50, 84], an event required for DR-induced apoptosis [85]. In contrast, catalytically active casp8 is detected within twenty-four hours of T cell receptor (TCR) ligation, but this is not associated with processed casp8. Moreover, casp8 processing appears to be dispensable for T cell clonal expansion [83]. Taken together, these studies lead to the conclusion that casp8 is activated in a specific fashion in response to DR vs. TCR ligation, a concept supported by the observation of unique post-translational modification of FADD and casp8 [83, 86] depending on the stimulus. However, the basis for this stimulus-dependent processing of casp8 remains poorly understood. Additionally, it is likely that the substrates targeted by casp8 following DR vs. TCR stimulation are distinct, an issue that requires additional clarification.

2.5 Autophagy

Cellular macroautophagy (herein referred to as “autophagy”) is a cellular process of self-eating, in which cytosolic macromolecules and organelles are engulfed by *de novo* generated autophagosomal membranes [87]. Following engulfment and generation of double membrane autophagosomes, these vesicles fuse with lysosomes, leading to the breakdown and recycling of macromolecules and damaged organelles. Lymphocytes are acutely dependent on autophagy, as loss of autophagy in B and T cells results in numerous defects in homeostasis and activation [88]. As autophagy is a key process to respond to intracellular stress and nutrient deprivation [87, 89], it is not surprising that it has been associated with different forms of cell death [90]. It is believed that autophagy may induce a unique form of cell death termed autophagic- or type-II cell death [91], and that this cell death occurs in a manner distinct from apoptotic death [92]. Supporting the concept that cell death and autophagy pathways are tightly interwoven, anti-apoptotic Bcl2 family members bind to Beclin-1, an important factor in the induction of autophagosome formation, and diminish autophagic flux [93].

Casp8 has also been found to modulate autophagy, as loss of casp8 in L929 cells results in heightened autophagy and an increase in type-II cell death [94]. In this study, loss of casp8 expression by virtue of siRNA mediated casp8 knockdown resulted in RIP1-dependent cell death (see below), implicating this caspase as an inhibitor of autophagic flux and associated type-II cell death [92]. FADD was also found to play a role in modulating autophagic signaling via direct binding to the autophagy regulator Atg5 [95]. Further tying DISC proteins to autophagy, cFLIP has been found to bind and inhibit Atg3 [96], an intermediate in the lipidation of the autophagosomal membrane protein LC3 [97]. Of note, T cells bearing defective FADD or casp8 function become hyperautophagic following TCR stimulation, and are susceptible to RIP1-dependent cell death following TCR stimulation [98, 99]. While it is unclear whether autophagy participates in the demise of T cells lacking FADD or casp8 signaling, necroptotic signaling has been definitively implicated. Jurkat T cells lacking

FADD have also been found to be highly susceptible to TNF-induced necroptosis [100], and blockade of RIP1 using necrostatin-1 (Nec-1) rescues these cells [101]. Moreover, Nec-1 eliminates the embellished autophagic flux observed in FADD-deficient Jurkats treated with TNF, suggesting that this hyperautophagy may be due to the cellular response to necroptosis.

2.6 Necroptosis

Programmed necrosis is a form of cell death that ensues in a distinct fashion from caspase-dependent apoptosis. Termed “necroptosis” [101], this process of cell death was initially described in the context of the casp8 deficient Jurkat cell line JB-6 treated with anti-Fas antibodies [102]. Vandenabeele and coworkers had observed similar non-apoptotic cell death in the context of L929 cells treated with the pan-caspase inhibitor Z-VAD-FMK, particularly after stimulation with TNF-alpha [16]. Subsequent studies by Tschöpp and colleagues revealed similar non-caspase dependent cell death in a FADD-deficient Jurkat line [100]. This group also demonstrated that the catalytic activity of the serine/threonine kinase RIP1 was required for this non-apoptotic cell death. Using knowledge of this requirement, Yuan and colleagues screened a series of chemical compounds to identify inhibitors of necroptosis [101], a screen resulting in the identification of several “necrostatins.” These include Nec-1, an allosteric inhibitor of RIP1 that potently inhibits TNF-alpha induced death of FADD-deficient Jurkats [17]. Hitomi et al. employed an siRNA screen to identify a large signaling network that distinguishes RIP1-dependent necroptotic vs. caspase-dependent apoptotic cell death [103], demonstrating that these death processes occur in very different ways.

While there are several distinct forms of non-apoptotic cell death, necroptosis as described here refers specifically to that cell death that requires RIP1 activity [104]. This form of death was also likely observed in the context of L929 cells treated with casp8 siRNA, since it was found that RIP1 was involved in this caspase-independent cell death associated with autophagy [94]. Blockade of RIP1 via Nec-1 or shRNA-mediated knock down led to the restoration of T cell proliferation in mice lacking FADD or casp8 activity, demonstrating that the proliferative defects observed in DISC-protein deficient T cells were likely due to necroptosis [98, 99, 105]. In addition to RIP1, a homologous serine/threonine kinase called RIP3 is also required for necroptosis [106–110]. Upon RIP1 activation, RIP3 is subsequently phosphorylated and binds to RIP1, leading to the formation of stable RIP1/RIP3 containing “necrosomes” [111] – see Fig 3. Substantiating the hypothesis that RIP1/RIP3-mediated necroptosis promotes the demise of DISC-protein deficient lymphocytes upon mitogenic stimulation, T cells lacking FADD or casp8 function were observed to proliferate normally when they bore a compound deficiency in RIP3 [112–116]. These observations demonstrate that, in the absence of normal DISC function, lymphocyte mitogenic signaling results in RIP1/RIP3 dependent necroptosis, although the basis for this remains to be established.

The means by which casp8 prevents the induction of necroptosis remains to be firmly established. RIP1 is subject to cleavage by active casp8, and this event was originally thought to block its ability to promote anti-apoptotic signaling via NF kappa B [117, 118].

RIP3 is similarly cleaved by casp8, resulting in a loss of kinase activity and necroptotic signaling [106]. Thus, processing of RIP1 and RIP3 by casp8 may prevent the elaboration of necroptotic signaling in wildtype T and B cells following their mitogenic stimulation. Supporting this hypothesis is the observation that antigenic stimulation of T cells leads to cleavage of both RIP1 and RIP3, conditions that failed to induce PARP1 cleavage and apoptotic cell death [115]. These findings support the hypothesis that casp8 inhibits the assembly or function of RIP1/RIP3 containing necrosomes through direct cleavage of these proteins. Lending further support to this hypothesis, RIP1-deficient Jurkat cells [119] bearing a casp8-resistant isoform of RIP1 were hypersensitive to TNF-alpha induced necroptosis. Confounding the identification of key proteolytic target(s) of casp8 acting in its survival role is the finding that RIPK1 is cleaved very efficiently during apoptosis, calling into question whether this is an important substrate of casp-8 [120]. An alternative explanation for the embellished necroptotic sensitivity observed in casp8-deficient cells has been raised by Ting and colleagues, observing that casp8-mediated cleavage of the E3 ubiquitin ligase CYLD enhances RIP1/RIP3 dependent necroptosis [121].

It is still far from clear whethercasp8 has numerous targets in the different necroptotic pathways that are subject to cleavage by this protease, thus determining the cell death mechanism under distinct conditions, or whether there is a key substrate yet to be identified, maybe different substrates for necroptotic events driven by divergent stimuli.

3. Conclusions - Missing links

Stimulus dependent necrosome formation

As described above, RIP1/RIP3 containing necrosomes are formed in mitogenically stimulated T cells lacking FADD or casp8 activity [115], although the basis for this is poorly understood. Moreover, the rescue of embryonic lethality in FADD- and casp8-deficient mice by a compound deletion of RIP3 suggests that casp8 activity is required in specific cell types during embryogenesis to prevent necroptosis [112–114]. While it is possible that necrosome formation in such cells occurs via DR signaling, this may not necessarily be the case. Indeed, blockade of DR signaling failed to rescue T cells lacking casp8 [83]. In addition, casp8 processing appears to occur distinctly upon antigenic- vs. DR-stimulation, supporting the hypothesis that casp8 itself is recruited to and activated by different intracellular scaffolds. This is supported by studies demonstrating that cFLIP binding promotes casp8 activation without interdomain cleavage [50], and that $FLIP_L$ prevents necroptosis [114, 122]. The potential that differentially processed casp8 may target distinct substrates is also an important issue to clarify.

Crosstalk between necroptosis and autophagy

Autophagy and RIP1/RIP3 dependent necroptosis are often observed to occur in tandem, suggesting that they are linked mechanistically [123]. The RIP1 inhibitor Nec-1 blocks the induction of hyper-autophagy in antigenically stimulated primary T cells [98] and TNF-alpha treated, FADD-deficient Jurkat cells [101]. Thus, the induction of necroptosis may lead to autophagy, perhaps in response to depletion of cellular energy stores during necroptosis [80]. Alternatively, since Nec-1 interferes with RIP1 activity allosterically, RIP1

may induce autophagy independent of its role in promoting necroptosis. Thus, it will be important to discern how autophagic and necroptotic signaling cascades are interwoven.

Does $FLIP_L$ activate or inhibit caspase-8?

Models proposing cFLIP as an inhibitor of DISC formation have been proposed several times, on the assumption that recruitment to the DISC of this inactive caspase paralog prevents casp8 or 10 recruitment. Yet as we have described, $FLIP_L$ but not $FLIP_S$ can activate casp8 and 10. Thus the relative concentrations of $FLIP_L$ and $FLIP_S$ are likely to define whether casp8 and 10 are activated or inhibited, and clear data on this are missing because most experimental data are derived from ectopic expression. Defining the conditions and situations, likely to be heavily cell-type dependent, that the different cFLIP forms use to influence caspase activity will probably be decisive in understanding death versus survival outcomes.

Caspase-8 survival substrates

A few proteins engaged in necroptotic signaling have been proposed as targets of casp8 (or casp8/FLIP) but there is as yet no consensus. Different reports implicate different proteins. Again, the key substrates may be cell-type dependent, there may be one key substrate, or there may be several. Emergent focused proteomic and genome editing tools are likely to be required to tease out this issue.

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Abbreviations

DED	Death Effector Domain
DISC	Death Inducing Signaling Complex
TNF	Tumor necrosis factor
TNFRI	Tumor necrosis factor receptor I
Z-VAD-FMK	benzoxycarbonyl-Val-Ala-Asp-fluoromethyl ketone

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Highlights

- Caspase-8 is required for death receptor apoptosis and, paradoxically, survival of some cell types
- This switch is requires protein kinases of the RIP family, which engage a pathway known as necroptosis
- The pro-apoptotic and pro-survival functions of caspase-8 are regulated by the pseudo-caspase cFLIP
- The mechanism of how caspase-8 and cFLIP coordinate the switch between apoptosis and survival is unknown
- The reason why an apoptotic initiator has been co-opted as a critical survival factor is unknown

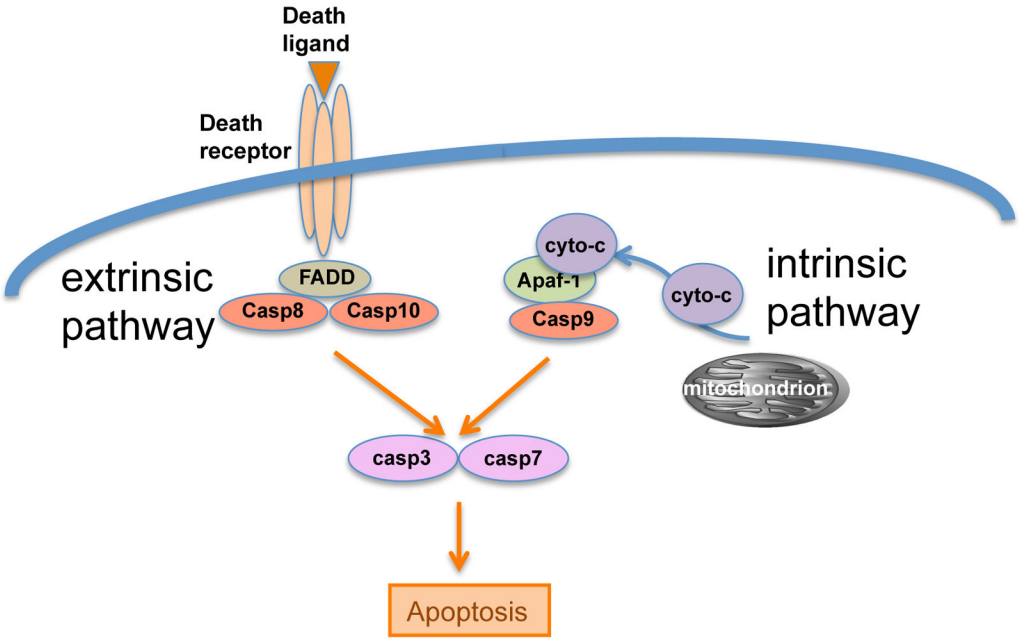


Figure 1. Extrinsic and Intrinsic Apoptosis Pathways

Extrinsic and intrinsic signals regulating apoptosis had been proposed in descriptive terms [12], and later revised to cover mechanistic events, such that death mediated by death receptors (DRs) became known as the extrinsic apoptosis pathway, and death mediated through mitochondria became the intrinsic apoptosis pathway [13]. The pathways activate distinct apical caspases (casp8 and 10 in the extrinsic pathway and casp9 in the intrinsic pathway), which converge on the activation of executioner caspases 3 and 7, leading to cleavage of an unknown number of proteins to express the apoptotic phenotype.

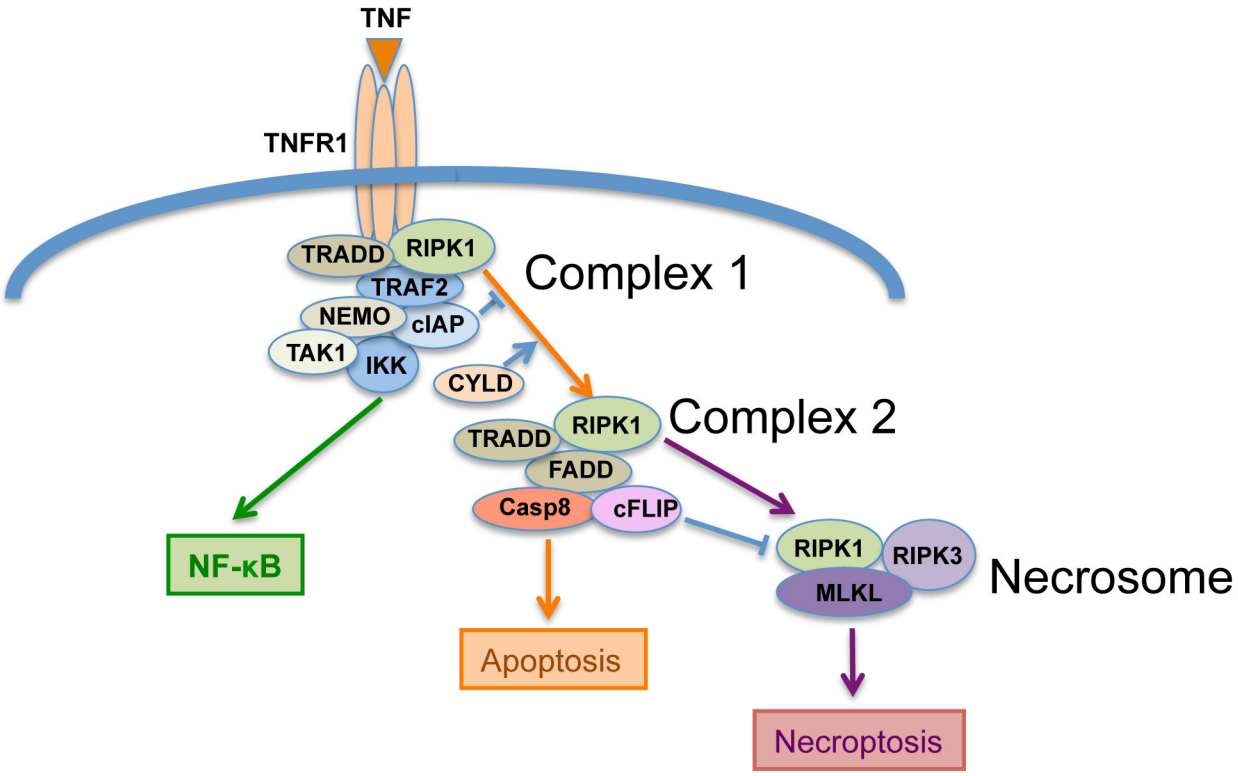
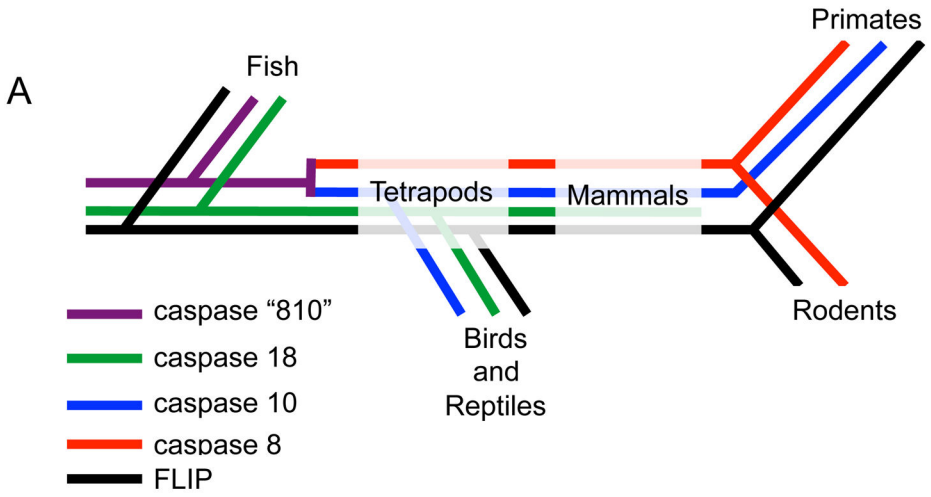


Figure 2. Pathways Emerging from TNFR1

Upon ligation of TNFR1 three pathways emerge to program different cell outcomes. NK-κB activation is triggered through the IKK complex, and depending on the concentration of available adaptors, a separate complex that recruits components of the DISC is engaged (Complex 1). Complex 2 is considered to be the main functional apoptotic protein cluster, but it is likely that a continuous range of adaptor and effector recruitments evolves, with complex 1 and complex 2 as the extremes. The Necrosome [19], synonymous with the Ripoptosome [20, 21], is engaged. The outcome of apoptosis versus necroptosis is controlled by casp8 and cFLIP.



B

	317	360 373	412	Uniprot
Human	CILSHGDKG	FFIQACQGDNYQKG-------IPVETDSEEQPYLEMDLSSPQTR	NCVSYRNPA	CASP8_HUMAN
Human	VLVSRGGSQ	FFIQNYVVSEGQLE-----DSSLLEVDGPAMKNVEFKAQKRGLC	MSLLEQSHS	CFLAR_HUMAN
Mouse	VLVSLGGSQ	FFIQNYESLGSQLE-----DS-SLEVDGPSIKNVDSKPLQPRHC	VSHLEKPSS	CFLAR_MOUSE
Dog	VLVSRGGSQ	FFIQNYVVSEGQMD-----SSSFLEVDGPEVNNVESKAKQPGPC	VSLLEQSSG	E2RR61_CANFA
Taz	IVISRGDSE	FFIQNYVVPKDHRE-----ANSLLEVDGGDGK-VSYHAWGPRSS	VSVLEEAPG	G3WLJ6_SARHA
Opossum	IVISRGNSE	FFIQNYMVAEDQQE-----SNNVLEVDGDGGK-GSYLGQRPGGS	VSVLERYPN	F7F390_MONDO
Chicken	ILVSRGNAQ	FFIQSYIVPEDEQE-----YT-SLEIDGNDKK-IISRAKTPLKD	VSTLEKSPR	E9NPB4_CHICK
Xenopus	IIISRGTSN	FFVQNYIAQEDEDL-----DK-MIEADGPTSAC-------AHSP	ERYLHQSPS	Q498I3_XENLA
Pufferfish	CIISRGTSN	LFIQRYNVAECQPHNWREHQDEDLETDG--FNG------HTTVD	EHQLEEGPH	H2U5F9_TAKRU
Zebrafish	CLISRGTNT	FFTQLYRITEAPVMP--SMDDEYLETDAPASRQ------CSNTG	VKLLEESGH	F1R706_DANRE

Figure 3. Caspase 8 and cFLIP phylogeny

Distinct family members related to modern casp8 arose in the chordate branch of the eukaryotes. A) Caspase 18 and the ancestor of caspases 8 and 10 (which we call “caspase 810” in this schematic, are still found in modern fishes. Later on in evolution, caspase 8 and 10 likely branched from caspase 810. Birds and lizards express three apical caspases in the DR pathway: caspase 8, 10 and 18. Mammals subsequently lost caspase 18, while rodents lost both caspase 10 and 18, leaving them with a single apical caspase of the extrinsic pathway. cFLIP is maintained throughout the lineage. See also Eckhart *et al.* [52]. B) Human casp8 (red) aligned with cFLIP paralogs. In the case of cFLIP, the conservation of mutated active site residues is sustained from fish to mammals (green highlight), as is the conservation of the cleavage site between the large and small subunits (yellow highlight). Each representative species seems to have only one cFLIP paralog, with the exception of the Japanese pufferfish that has two (but not surprising given the duplication of the pufferfish genome).