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## INHIBITOR OF APOPTOSIS PROTEINS AS INTRACELLULAR SIGNALING INTERMEDIATES

Andrew J. Kocab<sup>1,2</sup> and Colin S. Duckett<sup>2,3</sup>

<sup>1</sup>Graduate Program in Immunology, The University of Michigan, Ann Arbor, Michigan 48109

<sup>2</sup>Department of Pathology, The University of Michigan, Ann Arbor, Michigan 48109

<sup>3</sup>Department of Internal Medicine, The University of Michigan, Ann Arbor, Michigan 48109

### Abstract

The inhibitor of apoptosis (IAP) proteins have often been considered inhibitors of cell death due to early studies describing their ability to directly bind and inhibit caspases, the primary factors that implement apoptosis. However, a greater understanding is evolving for the vital roles played by the IAPs as transduction intermediates in a diverse set of signaling cascades that have been associated with functions ranging from the innate immune response to cell migration to cell cycle regulation. In this review, we discuss the functions of the IAPs in signaling, focusing primarily on the cellular IAP (c-IAP) proteins. The c-IAPs are important components in the TNF receptor superfamily signaling cascades, which include the activation of the NF- $\kappa$ B transcription factor family. Since these receptors can modulate cell proliferation and cell death, the roles of the c-IAPs in these pathways provide additional means of controlling cellular fate beyond simply inhibiting caspase activity. Additionally, IAP binding proteins, such as Smac and caspases, which have been described as having cell death-independent roles, may impact c-IAP activity in intracellular signaling. Collectively, the multifaceted functions and complex regulation of the c-IAPs illustrate the importance of the c-IAPs as intracellular signaling intermediates.

### Keywords

NF- $\kappa$ B; IAP; apoptosis; TNF; caspases

### Introduction

The study of cell death and its regulation led to the discovery and characterization of a family of factors called the inhibitor of apoptosis (IAP) proteins, and over the last few decades, the IAP protein family has been found to play major roles in a multitude of cellular processes. The mammalian IAP family consists of eight members, all of which share the family-defining baculovirus IAP repeat (BIR) domain (Fig. 1), and while every IAP member possesses at least one BIR domain, many members contain multiple BIRs. Three of

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Corresponding Author: Colin S. Duckett, Ph.D., NCRC Bldg 18, Rm 133, 2800 Plymouth Rd., Ann Arbor, MI 48109-2800; colind@med.umich.edu.

#### Author Contributions

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the most highly characterized IAPs are X-linked IAP (XIAP), cellular IAP 1 (c-IAP1), and cellular IAP 2 (c-IAP2), each of which possess three BIRs (Fig. 1) that are responsible for protein-protein interactions between the IAPs and other factors, such as caspases [1, 2]. In addition to the BIR motifs, XIAP and the c-IAPs contain a RING domain, which confers E3 ubiquitin ligase activity and plays an important role in the functional activities of these proteins (Fig. 1). The c-IAPs also contain two additional domains: a caspase-associated recruitment domain (CARD) and an ubiquitin-associated (UBA) domain (Fig. 1). While their exact functions remain unresolved, the CARD may allow the c-IAPs to interact with additional CARD-containing proteins and appears to regulate the E3 ubiquitin ligase activity by affecting the conformational status of the protein by interacting with the RING domain [3–5], and the UBA domain has been shown to bind ubiquitin and has recently been found to facilitate recruitment of other factors involved in the ubiquitination process [6–8].

Early studies with the IAPs were based on the notion that the IAPs regulate cell death through the inhibition of caspases. XIAP is the archetypical IAP protein that directly binds and inhibits caspases, specifically caspases-3, -7, and -9, preventing their downstream effector functions [9, 10]. This binding occurs through the BIR domains of XIAP and has been shown to inhibit cell death [11, 12]. Due to this described role, members of the IAP protein family have been generally considered direct inhibitors of cell death. The caspase inhibition exhibited by XIAP is suppressed following the initiation of apoptotic pathways that progress through the mitochondria. During apoptosis, the mitochondria is permeabilized during a process called mitochondrial outer membrane permeabilization (MOMP), releasing mitochondrial proteins into the cytoplasm, including Smac and the serine protease Omi/HtrA2, that possess IAP binding motifs (IBMs) and bind to XIAP in a manner that liberates the caspases, allowing them to be activated (Fig. 2) [13].

Smac is capable of binding c-IAP1/2 and inducing their autoubiquitination and degradation [14–18]. However, despite their ability to bind caspases, the c-IAPs do not possess the ability to inhibit the apoptotic functions of caspases, unlike XIAP [19]. This suggested that the c-IAPs might regulate cell death through other mechanisms. Recent work has implicated the c-IAPs in regulating other cell death-activating platforms. Stimulation of tumor necrosis factor-receptor 1 (TNF-R1) following the removal of the c-IAPs by chemical, physiological, or genetic means, results in the formation of a complex containing FADD, active caspase-8, and receptor-interacting serine/threonine protein kinase 1 (RIP1) to induce apoptosis [20]. Additionally, the c-IAPs have been associated in a recently described form of cell death, designated necroptosis, which exhibits hallmarks of both apoptosis and the inflammation-inducing necrosis and occurs following the chemical degradation of the c-IAPs in conjunction with the inhibition of caspases [21, 22].

Recently, it has become more apparent that the IAPs play important functions in a host of cellular processes beyond caspase and cell death inhibition, implying that caspase-binding may, perhaps, only represent a minor facet of the IAPs. As such, the IAPs have come to be recognized as important regulators of intracellular signaling cascades, specifically the activation of nuclear factor- $\kappa$ B (NF- $\kappa$ B). The c-IAPs, as well as XIAP, have been implicated in multiple pathways of NF- $\kappa$ B activation [23], a family of transcription factors that play vital roles in a variety of signaling relevant to immunology and cancer as will be described

in more detail below. Even though they are poor inhibitors of caspase activity, the c-IAPs may contribute to the determination of cell fate through their regulation of NF- $\kappa$ B.

## IAPs as Regulators of Cell Signaling

As mentioned above, the functional scope of the IAPs has expanded beyond caspase-binding and cell death inhibition. The IAPs have been implicated in a diverse spectrum of non-apoptotic signaling, ranging from the regulation of intracellular copper levels [24–26] to the control of cell cycle progression [27, 28] and cell migration [29, 30]. Additionally, IAPs are important signal transduction factors in a variety of receptor-mediated pathways, many of which activate NF- $\kappa$ B [20].

As discussed above, several members of the IAP family, including the c-IAPs and XIAP, possess RING domains, which have been shown to be vital in many of the signaling cascades in which the IAPs participate. In these signaling pathways, the IAPs, through their RING domains, function as E3 ubiquitin ligases, which are the final component in the ubiquitination enzymatic cascade. The consequences of two polyubiquitin chains, the K63-linked chain and the K48-linked chain, have been well studied. These polyubiquitin chains are defined by their linkages at specific lysines within the ubiquitin protein [31], and the IAPs have been shown to mediate both K48 and K63 ubiquitination [32, 33]. The K48 ubiquitin chain is generally considered to be a degradation signal that targets the marked protein for proteasome-mediated destruction. In contrast, K63 polyubiquitination generally does not mediate proteasomal degradation, but can act as a scaffold for recruitment of additional factors to the signaling complex [34]. The c-IAPs are E3 ubiquitin ligases for multiple components in the NF- $\kappa$ B pathways, including RIP1 and NF- $\kappa$ B-inducing kinase (NIK), and the ability to mediate these forms of ubiquitination is integral to the functions of the IAPs in regulating the activation of NF- $\kappa$ B [18, 23].

NF- $\kappa$ B is a family of transcription factors that plays important functions in many cellular responses. The family is comprised of five members: p65 (RelA), RelB, c-Rel, p50 (NF- $\kappa$ B1), and p52 (NF- $\kappa$ B2), and following activation, these factors dimerize to form the active transcription factor [35]. Prior to activation, NF- $\kappa$ B dimers are sequestered in the cytoplasm of the cell, rendered inactive through the binding of I $\kappa$ B proteins. These proteins mask the nuclear localization sequence in NF- $\kappa$ B and prevent DNA binding through their ankyrin repeats [36]. Initiation of the signaling cascade results in I $\kappa$ B $\alpha$  ubiquitination and subsequent degradation by the proteasome following, allowing the NF- $\kappa$ B dimer to translocate into the nucleus.

Two major pathways of NF- $\kappa$ B signaling have been described: the canonical pathway that involves the p65:p50 dimer and the non-canonical pathway that employs the RelB:p52 dimer [35]. Canonical NF- $\kappa$ B can be activated by a wide variety of stimuli, some of which involve the IAPs, and the resulting signaling cascades converge on the I $\kappa$ B kinase (IKK) complex [37]. This complex has three components: IKK $\alpha$  (IKK1), IKK $\beta$  (IKK2), and NF- $\kappa$ B essential modulator (NEMO, also known as IKK $\gamma$ ). IKK $\alpha$  and IKK $\beta$  are the kinase subunits of the complex, while NEMO is a regulatory factor [38]. Once activated, the IKK complex phosphorylates I $\kappa$ B $\alpha$ , triggering its subsequent ubiquitination and proteasome-

mediated degradation. As described above, this allows the canonical NF- $\kappa$ B dimer to translocate to the nucleus and initiate transcription. Non-canonical NF- $\kappa$ B, which is activated by a more limited set of stimuli, relies on a protein called NF- $\kappa$ B-inducing kinase (NIK). Prior to stimulation, NIK is constitutively degraded by a c-IAP-containing complex [39]. Following activation, this complex is inhibited, resulting in an increase in NIK protein levels, which then leads to the activation of IKK $\alpha$ . IKK $\alpha$  phosphorylates the NF- $\kappa$ B precursor protein p100 at Ser-866 and Ser-870 [36], resulting in its proteasome-dependent cleavage and generation of the active p52 subunit. The RelB:p52 dimers can then translocate into the nucleus to modulate gene expression. While the IAPs have been shown to participate in a variety of NF- $\kappa$ B activation pathways, the c-IAPs have been implicated in the regulation of both canonical and non-canonical NF- $\kappa$ B, playing both activating and inhibitory roles [20].

## IAPs and the TNF Receptor Superfamily

These contrasting regulatory roles are evident in the signaling cascades of the TNF receptor superfamily, which can be broadly characterized into two major subsets based on their structural domains. Receptors such as Fas and TNF-R1, which can be widely expressed, possess death domains within their cytoplasmic tails [40]. These domains allow the receptor to recruit other DD-containing factors, building a receptor complex that can induce a multiple signaling cascades. These receptors have important roles in inflammation, hematopoiesis, and the immune response [34]. In contrast, certain TNF receptor superfamily members, such as CD30 and CD40, do not possess death domains and instead recruit signaling cofactors via TRAF-binding domains. These domains allow for the recruitment of members of the TNF receptor-associated factor (TRAF) family [20], and while TRAFs are implicated in signaling from both groups of receptors, direct TRAF binding to the receptor results in distinct consequences [41]. Additionally, the expression of these receptors is more limited, and many of these receptors are predominantly found on hematopoietic cells [42], and as such, many of these receptors have been shown to play important roles in immunity.

The c-IAPs are important factors in signaling from both categories of the TNF receptor superfamily, playing distinct roles for each subset of the superfamily. In the context of NF- $\kappa$ B activation by TNF-R1, a DD-containing receptor, the c-IAPs actively participate in the recruitment of proteins for signal transduction. Upon ligand binding to TNF-R1, the adaptor molecule TNF-R1-associated death domain protein (TRADD) is recruited to the DD of TNF-R1 [43]. TRADD binding, in turn, recruits additional proteins, including TRAF2, c-IAP1/2, and RIP1 to form the initial receptor signaling complex (Fig 3) [44, 45]. The c-IAPs become polyubiquitinated in a K63-dependent manner through autoubiquitination and polyubiquitinate RIP1, resulting in K63- and K11-linked ubiquitin chains that form a scaffold for the recruitment of additional factors, including transforming growth factor- $\beta$  activating kinase 1 (TAK1), TAK1 binding protein 2 (TAB2), and TAB3, which preferentially binds to K63-linked ubiquitin chains (Fig 3) [32–34, 46, 47]. Additionally, the tripartite linear ubiquitin chain assembly complex (LUBAC), comprised of Sharpin, HOIL-1, and HOIP, is recruited to the signaling complex [34, 48, 49]. LUBAC modifies the NEMO subunit of the IKK complex with linear ubiquitin chains, which is believed to impact

its function [49]. IKK $\beta$  is phosphorylated by the TAK:TAB complex, activating the IKK complex and resulting in the subsequent downstream activation of NF- $\kappa$ B (Fig 3) [50, 51].

While the E3 ubiquitin ligase activity of the c-IAPs is involved in the propagation of the TNF-R1 signaling cascade, the c-IAPs also play an inhibitory role in regulating non-canonical NF- $\kappa$ B in the context of CD30-mediated signaling. Prior to receptor activation, the c-IAPs form a complex with TRAF2 and TRAF3. This complex binds NIK, which is constitutively expressed [52, 53]. Both TRAF2 and TRAF3 have been shown to directly bind NIK [54, 55], and the c-IAPs modify NIK with K48-linked ubiquitin chains, resulting in its proteasome-dependent degradation [39, 41, 52, 56], thereby repressing non-canonical NF- $\kappa$ B activation (Fig. 4). Following receptor activation, the TRAF:c-IAP complex is recruited to the cytoplasmic tail of CD30 via its TRAF-binding domains. After this recruitment, the c-IAPs modify TRAF3 with K48-linked ubiquitin chains, leading to the degradation of TRAF3 (Fig. 4) [39, 52, 57]. TRAF2 and c-IAP1/2 are then subsequently degraded following translocation to a detergent insoluble fraction [18, 20, 57–59]. The absence of the c-IAP:TRAF complex allows NIK levels to accumulate, resulting in the activation of non-canonical NF- $\kappa$ B (Fig. 4). This signaling pathway highlights receptor-mediated degradation of the c-IAPs as an important regulatory event for the activation of non-canonical NF- $\kappa$ B.

## IAPs and Smac Mimetics

Receptor-mediated degradation of the c-IAPs can be partially mimicked by a class of small molecule compounds based on the IAP binding motif (IBM) of Smac [60–62]. These Smac mimetics (SMs), which are also known as IAP antagonists, similarly bind the IAPs and induce their degradation [56, 63, 64]. Due to the connection between the IAPs and cell death, SMs have been actively studied as potential therapeutics against cancer. While the ability of SMs to liberate caspases from XIAP may contribute to the observed induction of cell death, this is now believed to represent only one aspect of their mechanism. SMs have been shown to preferentially target and degrade the c-IAPs over XIAP [56, 65], and while this does not preclude the inhibition of XIAP, it suggested a more prominent role for the c-IAPs in regulating SM-induced cell death. It is currently thought that SM-induced killing is dependent on TNF [56, 63].

More specifically, following SM treatment, the c-IAPs are degraded, and TNF is produced in an autocrine or paracrine manner, activating TNF-R1. However, in the absence of c-IAP1/2, the ubiquitin scaffolds that help form the receptor signaling complex cannot be properly formed, leading RIP1 to then associate with TRADD, FADD, and caspase-8 to form a death inducing signaling complex, resulting in caspase activation and cell death [32]. Currently, cells can be divided into two major categories: those that are killed by SM treatment alone, and cells that are not killed by SM treatment alone but are sensitized to exogenous TNF [22, 66]. Cells in the former category are able to produce autocrine TNF through a NF- $\kappa$ B-dependent manner [66], while cells in the latter category, conversely, do not produce TNF following SM treatment, and therefore require an additional source of TNF [63]. More recently, another mechanism of cell death has been proposed which involves the formation of the ripoptosome following SM treatment. The formation of the ripoptosome,

which is comprised of RIP1, FADD, inactive caspase-8, and RIP3, a related protein to RIP1, is thought to occur in specific cases following genotoxic stress, certain inflammatory stimuli, and c-IAP degradation [21, 22, 67]. Importantly, ripoptosome-mediated cell death is independent of TNF and the mitochondrial death pathways [21, 22], indicating that SM-induced death may not always be reliant on TNF. Collectively, these data illustrate alternative means by which the c-IAPs may inhibit cell death, inhibition that is dependent on their roles in cell signaling rather than through their caspase binding.

In addition to the clinical relevance of studying SMs as potential anti-cancer therapeutics, these compounds may also provide insight into the regulation of the c-IAPs. As noted above, these compounds were based on Smac, a physiological binding partner for the IAPs. In standard models, Smac is sequestered in the mitochondria and is released during mitochondria-mediated cell death. However, recent work has described non-lethal form of mitochondria permeabilization, a process called minority MOMP [68]. During this event, a minority of mitochondria are permeabilized, leading to a limited and sub-lethal activation of downstream caspases. The activation of these caspases results in DNA damage, as well as the promotion of oncogenic transformation [68]. In addition to caspase activation, Smac release was also observed during this process [68]. While the exact role of Smac released during minority MOMP is unclear, Smac may be acting as a signaling cofactor resulting in undefined consequences. In general, this may suggest that novel non-apoptotic functions exist for Smac and other apoptosis-associated proteins, similarly to growing number of non-apoptotic roles for the IAPs that have been described. Furthermore, this may change how we view the relationship between the IAPs and their mitochondrial protein targets. The IAPs may act as sentries to bind and eliminated these proteins, preventing effects caused by their limited release from the mitochondria. Conversely, the mitochondrial proteins may be released in a non-apoptotic manner in order to bind and induce the degradation of the IAPs, thereby regulating their activity. Notably, a similar system has been described for regulating *Drosophila* IAP activity [69]. These ideas illustrate potential physiological regulatory networks that control IAP activity.

In addition to considering novel means of regulating the IAPs, their overall functional scope may need to be expanded. Recently, we examined the downstream consequences of physiological and synthetic IAP antagonism using transcriptome profiling, identifying potentially novel roles for the c-IAPs [70]. More specifically, we described a role for the c-IAPs in regulating the ribosome and protein synthesis [70]. IAP antagonism by both receptor activation and SM treatment resulted in decreased expression of genes encoding ribosomal proteins, which led to an overall inhibition of protein synthesis. Extrapolating from these results, the c-IAPs may affect sensitivity to cell death by modulating cell cycle progression, since protein synthesis inhibition has been previously associated with both cell cycle arrest [71–73] and increased sensitivity to certain death ligands, such as TRAIL [74, 75]. In addition to highlighting new facets of IAP function, these results may have clinical implications. As described above, SMs are being tested for their therapeutic value and induce a TNF-dependent death in certain cells [56, 63]. These results potentially suggest an additional mechanism of SM killing, wherein SM-induced c-IAP degradation triggers the shutdown of protein synthesis, mimicking the effects of cycloheximide and rendering the



cell more susceptible to TNF lethality. The lethality of TNF would then be partially due to the inability to synthesize pro-survival proteins, similar to established mechanisms of TNF killing [67, 76, 77]. Alternatively, c-IAP regulation of protein synthesis may affect sensitivity to cell death by inducing cell cycle arrest and increasing overall sensitivity to death.

These recently defined functions for the c-IAPs, along with the potential regulation of IAP activity by Smac released during a non-apoptotic MOMP event, represent intriguing new avenues of focus and warrant future study. It remains unclear if these effects are isolated to specific stimuli or if the c-IAPs engage in these functions in resting conditions. If the c-IAPs modulate ribosomal gene expression and protein synthesis under normal conditions, Smac could be released through a minority MOMP event to refine this effect. Furthermore, continued analysis of the transcriptome data may reveal more non-traditional roles for the IAPs.

While recent work expands the functional scope of the IAPs, their described role as caspase binding proteins may also be reevaluated. Caspases, though traditionally associated with cell death, have been increasingly implicated in non-death related functions. For example, in humans, caspases-1, -4, -5, and -12 are considered inflammatory caspases and are involved in the innate immune response [78]. Caspase-1, more specifically, plays an important role in the processing and maturation of IL-1 $\beta$  during inflammation [79, 80]. Additionally, the apoptotic caspases have also been shown to possess apoptosis-independent functions. For example, caspase-3 plays a role in cell proliferation and differentiation, which may stem from its ability to regulate Akt phosphorylation [78, 81]. Caspase-8, an important factor in apoptosis, has been implicated in the regulation of inflammation in a manner that is independent of apoptosis [82–84], and has been shown to participate in the differentiation of monocytes into macrophages through the regulation of NF- $\kappa$ B via cleavage of RIP1 [85, 86]. Additionally, we found that gene expression regulated by c-IAP degradation was dependent on caspases, illustrating another non-apoptosis-related function for these proteins [70]. These data may broaden the functional scope of the IAP:caspase interaction beyond regulation of cell death, with the IAPs acting as general regulators of caspase activity. More specifically, if the IAPs bind and functionally inhibit the caspases, minority MOMP may represent a regulatory event. Minority MOMP could occur, releasing Smac into the cytoplasm, which would then bind and degrade the IAPs, liberating the caspases to perform downstream functions (Fig. 5). As described above, minority MOMP is a recently described event that results in the sub-lethal activation of caspases, and this process also releases the IAP binding protein Smac [68] potentially representing another regulatory function. Therefore, the IAPs, through their caspase-binding ability, may regulate caspases in a variety of biological functions.

## Concluding Remarks

Our understanding of the biological function of the IAPs has expanded beyond their ability to directly bind and inhibit caspase activity, with their functions now encompassing major regulatory roles in a wide range of intracellular signaling pathways. The list of their cellular roles continues to expand as studies implicate the IAPs in a growing number of signaling

cascades. Similarly, reexamination of IAP binding proteins, such as Smac and caspases, and their relationships with the IAPs will be warranted as new functions for these proteins emerge. Elucidating the interplay between the IAPs, their binding partners, and their novel functions will expand our understanding of the multifaceted nature of the IAPs as well as the biological impact on their respective signaling cascades.

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## Abbreviations

<b>IAP</b>	inhibitor of apoptosis
<b>XIAP</b>	X-linked inhibitor of apoptosis
<b>c-IAP</b>	cellular inhibitor of apoptosis
<b>NF-<math>\kappa</math>B</b>	nuclear factor- $\kappa$ B
<b>TNF</b>	tumor necrosis factor

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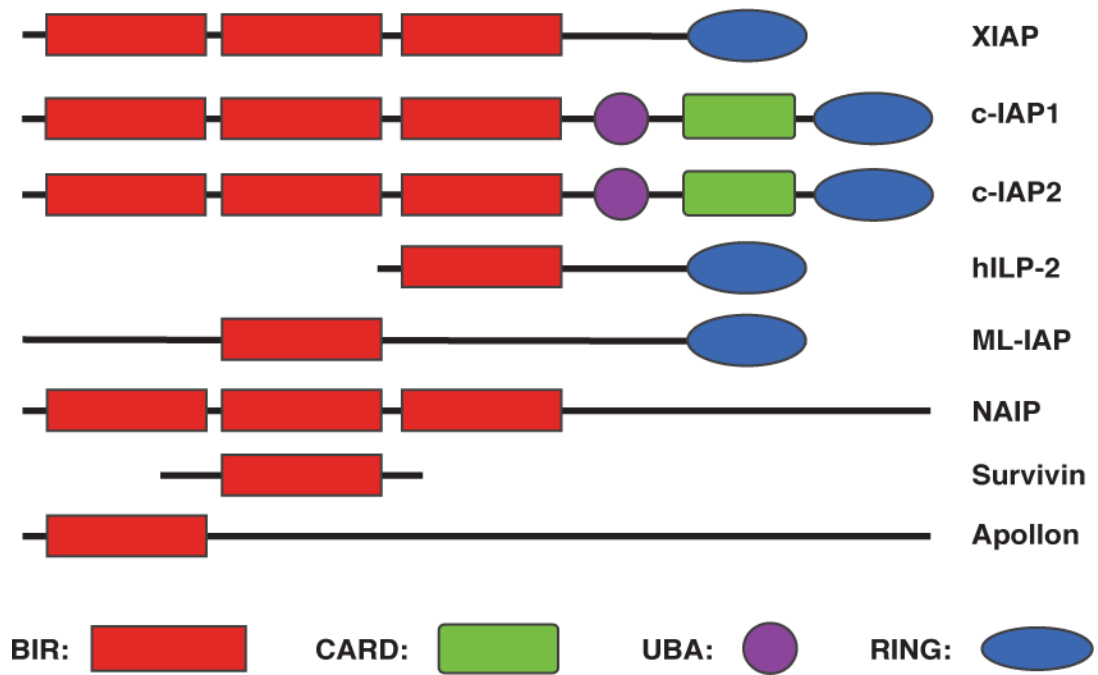
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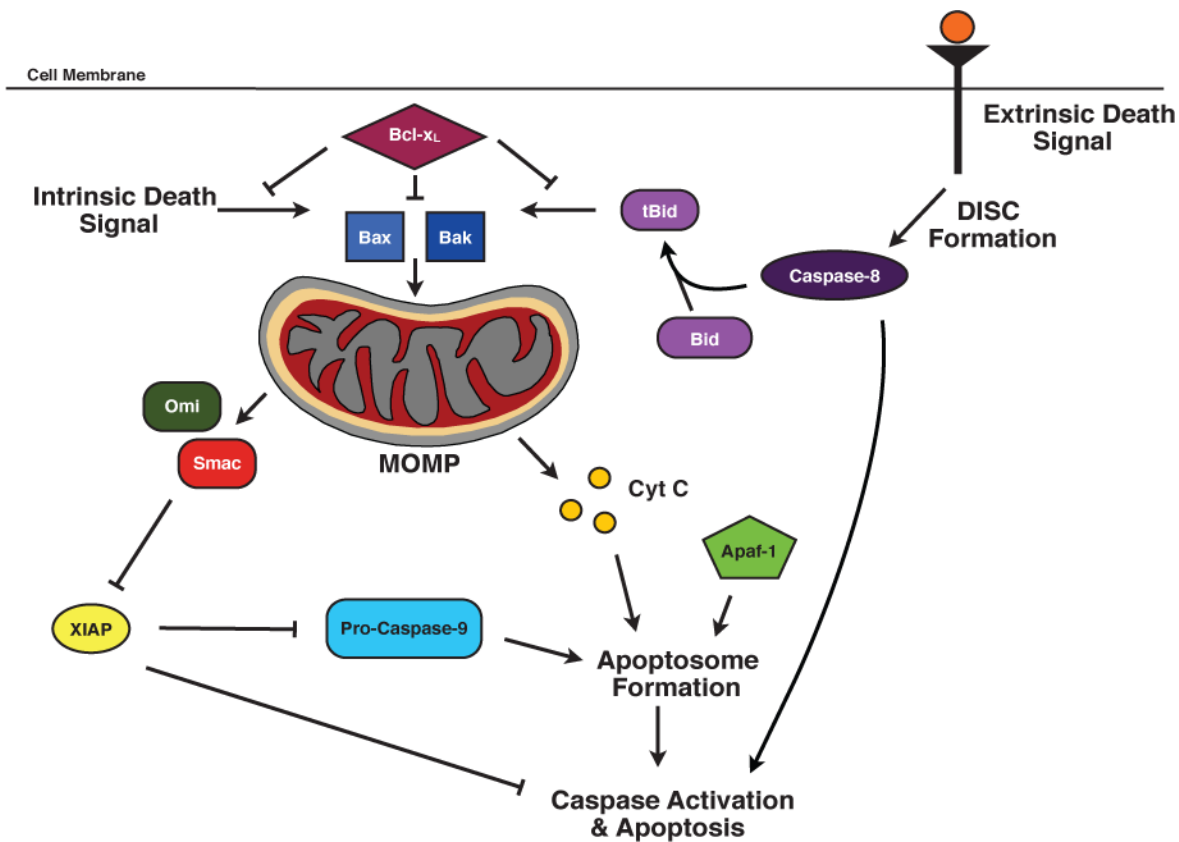
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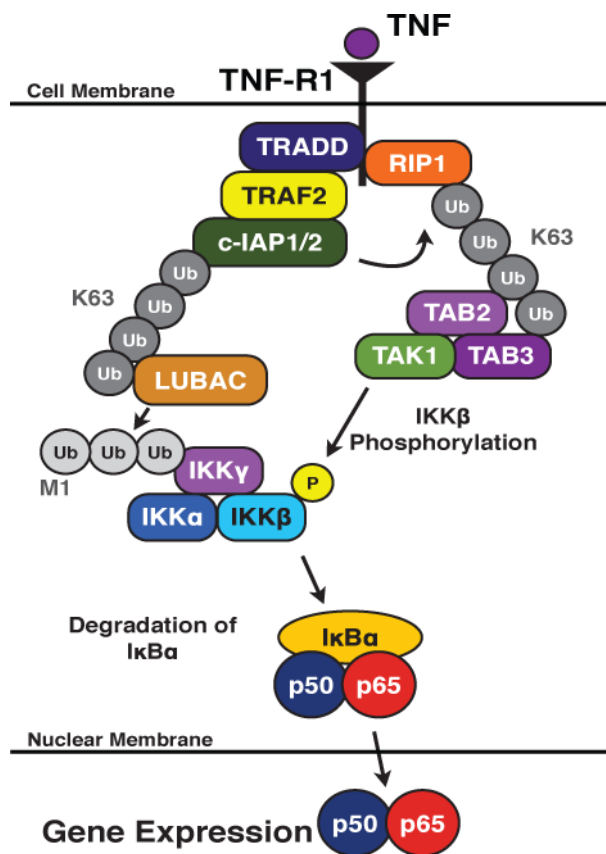


**Figure 1. Schematic depiction of the inhibitor of apoptosis (IAP) protein family**  
 BIR, baculovirus IAP repeat; CARD, caspase-associated recruitment domain; UBA, ubiquitin-associated domain; RING, really interesting new gene.



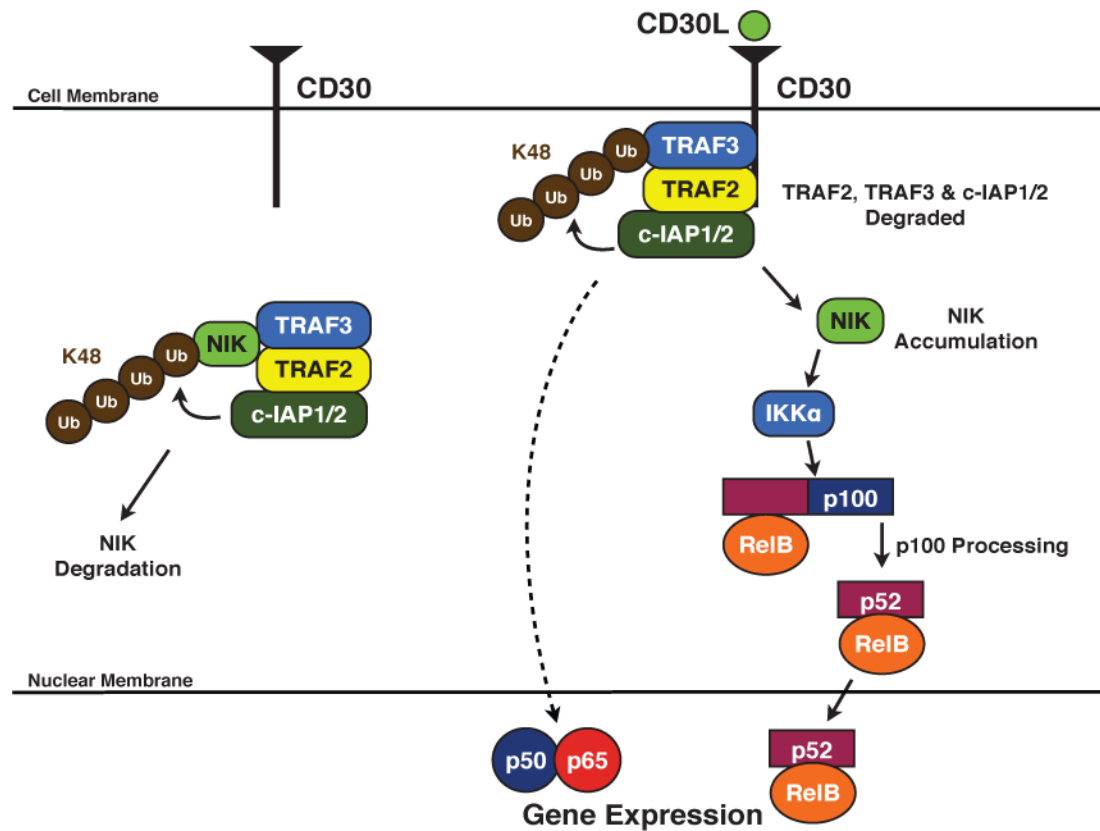


**Figure 2. The intrinsic and extrinsic apoptotic pathways**  
 The intrinsic cell death pathway is initiated by an intracellular death signal. This signal results in the oligomerization and translocation of Bax and Bak into the outer membrane of the mitochondria. This triggers mitochondrial outer membrane permeabilization (MOMP) and the release of cytochrome c and IAP binding proteins. Cytochrome c forms the apoptosome with Apaf-1 and pro-caspase-9, which results in cell death. The IAP binding proteins, such as Smac, bind to XIAP and antagonize the caspase-binding function of XIAP. The extrinsic cell death pathway is receptor-mediated and results in the formation of the death inducing signaling complex (DISC). In certain cells, the DISC can directly activate downstream caspases, leading to cell death. However, in most cells, the DISC, through its caspase-8 component, cleaves Bid to form tBid, leading to Bax/Bak oligomerization, MOMP, apoptosome formation, and subsequent cell death.



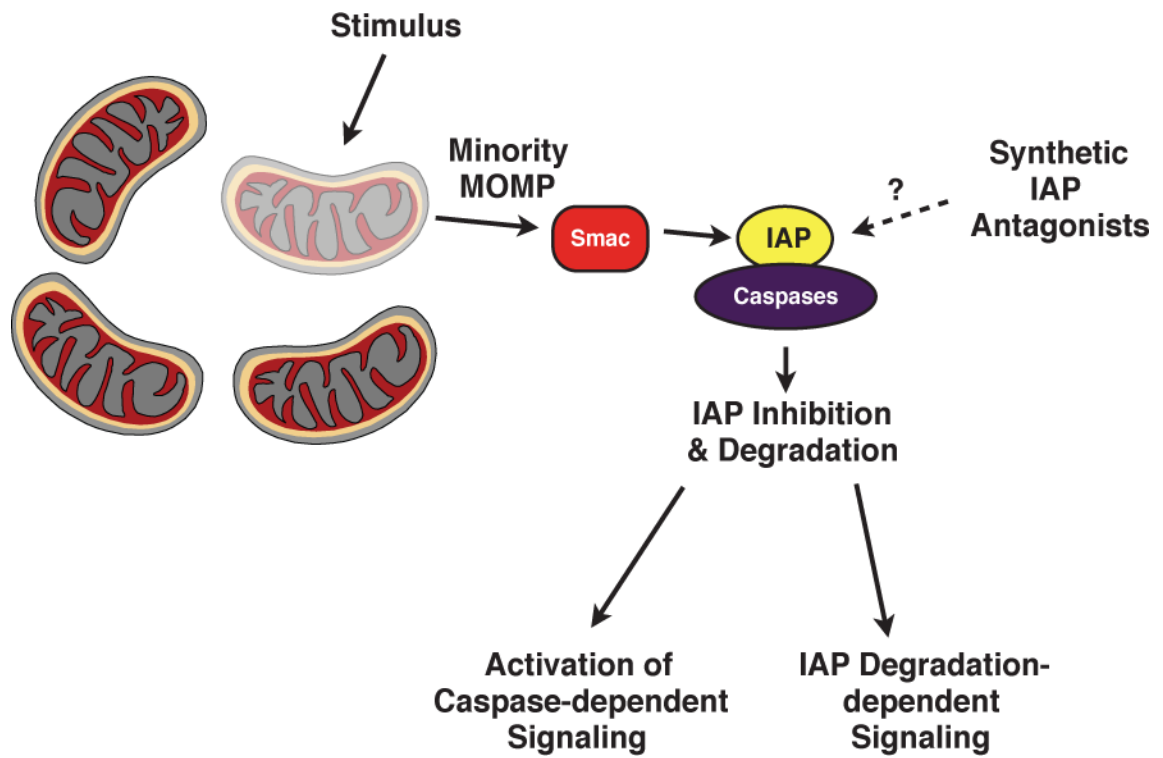
**Figure 3. The role of c-IAP1/2 in TNF-R1-mediated NF-κB activation**

Upon ligand binding to TNF-R1, death domain-containing factors, such as RIP1 and TRADD are recruited to the cytoplasmic tail of the receptor. This, in turn, recruits additional factors, including TRAF2 and the c-IAPs. The c-IAPs undergo autoubiquitination and ubiquitinate RIP1. The ubiquitin chains act as scaffolds to recruit LUBAC and the TAB:TAK complex. The IKK complex is modified with a linear ubiquitin chain by LUBAC and is activated by phosphorylation by the TAB:TAK complex. The IKK complex phosphorylates IκBα, resulting in its subsequent proteasome-dependent degradation and the translocation of the canonical NF-κB dimer into the nucleus.



**Figure 4. The role of c-IAP1/2 in CD30-mediated NF-κB activation**

Prior to stimulation, the c-IAPs form a complex with TRAF2 and TRAF3 and ubiquitinate NIK in a K48-dependent manner, resulting in the constitutive degradation of NIK. Following receptor activation, the TRAF:c-IAP complex is recruited to the cytoplasmic tail of CD30, where the c-IAPs ubiquitinate TRAF3, inducing its degradation. TRAF2 and c-IAP1/2 are also degraded, allowing for the accumulation of NIK. NIK activates IKK $\alpha$ , which phosphorylates p100. Subsequently, p100 is cleaved, allowing the active non-canonical NF- $\kappa$ B dimer to translocate into the nucleus. Receptor-mediated canonical NF- $\kappa$ B activation also occurs, albeit through a poorly defined mechanism.



**Figure 5. Model of IAP regulation of caspase-dependent signaling**

Prior to stimulation, IAPs are bound to caspases, functionally inhibiting them. A stimulus triggers minority MOMP, resulting in the limited release of Smac into the cytoplasm. Smac binds, inhibits, and induces the degradation of the IAPs. The liberated caspases proceed to participate in downstream functions, while additional IAP degradation-dependent signaling pathways are activated in parallel. Synthetic IAP antagonists, or Smac mimetics, may replicate the role of Smac and induce this process in a MOMP-independent manner.