

Activation of Initiator Caspases: History, Hypotheses, and Perspectives

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Apoptosis is executed by a cascade of caspase activation. Activation of an effector caspase is mediated by specific initiator caspases through an intra-chain cleavage. Activation of an initiator caspase, such as caspase-9, relies on a specific adaptor protein complex, such as apoptosome. Although conclusive mechanisms by which the initiator caspases are activated remain elusive, important insights have emerged from recent investigations. The induced proximity model summarizes the general process of initiator caspase activation. The proximity-driven dimerization model describes how initiator caspases respond to induced proximity and offers an explanation for their activation. However caspases are activated, enhanced activity must be correlated with altered active site conformation. The induced conformation model posits that the activated conformation for the active site of a given initiator caspase is attained through direct interaction with the adaptor protein complex or through homo-oligomerization facilitated by the adaptor protein complex.

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Introduction

Apoptosis, the prevalent form of programmed cell death, plays a central role in the development and homeostasis of all multi-cellular organisms [1-4]. There are two well-characterized apoptotic pathways in cells. The extrinsic pathway, exemplified by the elimination of unwanted cells during animal development, is initiated by ligand-induced activation of the death receptors at the plasma membrane [5-7] (Figure 1). The intrinsic cell death pathway, on the other hand, is triggered by cellular stress signals such as DNA damage [4] (Figure 1). Alterations in apoptotic pathways have been implicated in many debilitating diseases in humans, including cancer, autoimmune diseases, and developmental and possibly neuro-degenerative disorders [8-12].

The mechanism of apoptosis is remarkably conserved across species, involving a cascade of sequential activation of initiator and effector caspases, cysteine proteases with aspartate substrate specificity [13-17]. Although the first cas-

pase, interleukin 1 β -converting enzyme (ICE²; or caspase-1), was identified in humans [18,19], the critical involvement of caspase in apoptosis was discovered in the nematode *Caenorhabditis elegans*, in which the indispensable gene *ced-3* was found to encode a cysteine protease and closely resemble the mammalian ICE [20,21]. Since then, at least 14 distinct mammalian caspases have been identified, with 11 from the human genome [22]. Seven mammalian caspases, including four initiator and three effector caspases, are known to play important roles in apoptosis (Figure 2). An initiator caspase is characterized by an extended amino-terminal region, which comprises one or more adaptor domains that are important for its function, whereas an effector caspase usually contains 20–30 residues in its prodomain sequence (Figure 2).

All caspases are produced in cells as catalytically inactive zymogens. The activation of an effector caspase (also known as executioner caspase), such as caspase-3 or caspase-7, is executed by an initiator caspase (also known as apical caspase), such as caspase-9, through proteolytic cleavage after a specific internal Asp residue to separate the large and small subunits of the mature caspase [13]. As a consequence of the intra-chain cleavage, the catalytic activity of an effector caspase is enhanced by several orders of magnitude [23]. Once activated, the effector caspases are responsible for the proteolytic degradation of a broad spectrum of cellular targets that ultimately lead to cell death [13].

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²Abbreviations: ICE, interleukin 1 β -converting enzyme; DISC, death-inducing signaling complex; Apaf-1, apoptotic protease-activating factor-1; CARD, caspase-recruitment domain; PIDD, p53-induced protein with a death domain; DD, death domain; FADD, Fas-associated death domain; DED, death effector domain; FKBP, FK506-binding proteins.

Figure 1: A schematic diagram of two well-characterized apoptosis pathways. The extrinsic pathway is initiated by ligand-induced activation of the death receptors at the plasma membrane, ultimately resulting in the activation of caspase-8 or caspase-10. The intrinsic cell death pathway is triggered by cellular stress signals such as DNA damage, culminating in the activation of caspase-9. Caspase-8 and caspase-9 are the initiator caspases; they cleave and activate the effector caspases, such as caspase-3 and caspase-7, which kill a cell by cleaving a wide range of protein substrates.

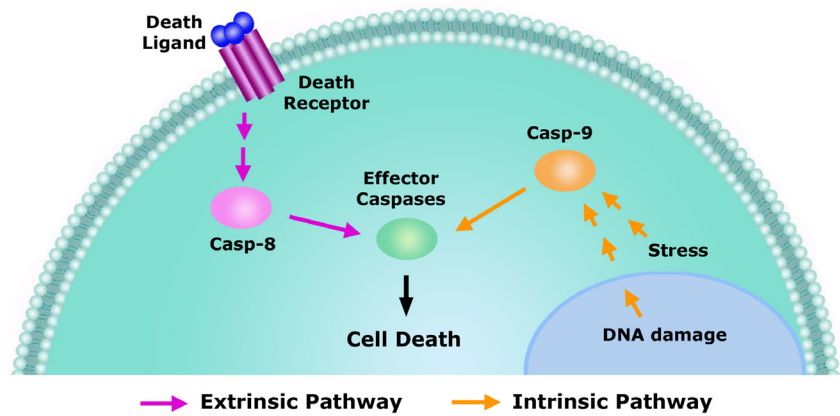
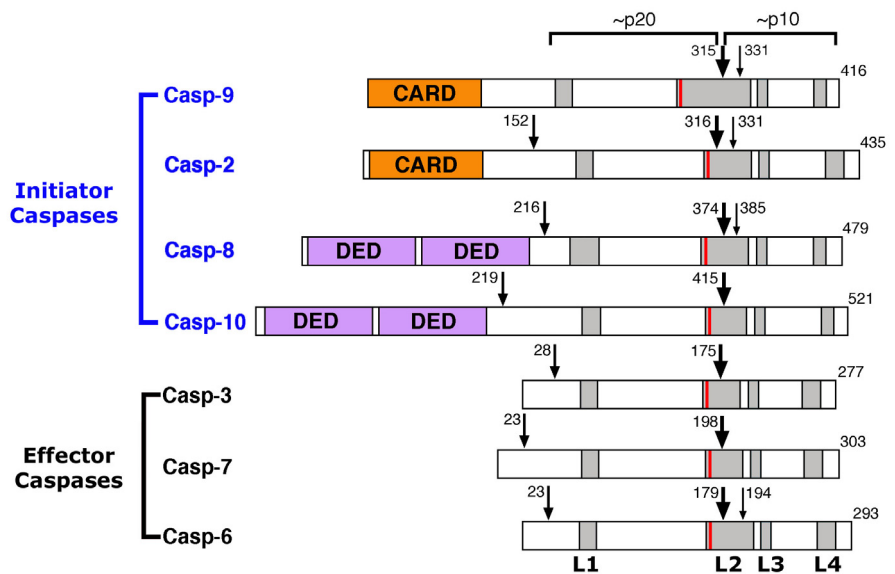


Figure 2: Apoptotic caspases in mammalian cells. Four initiator caspases and three effector caspases are drawn to scale. The position of the first intra-chain cleavage (between the large and small subunits) is highlighted by a large arrow whereas additional sites of cleavage are represented by medium and small arrows. The prodomains in initiator caspases invariably contain homotypic interaction motifs, such as the caspase recruitment domain (CARD) and the death effector domain (DED) [92]. The four surface loops, labeled L1 through L4, form an active site [22]. The catalytic cysteine residue is shown as a red line at the beginning of loop L2. The p20 and p10 subunits together form a caspase monomer.



Mechanism of activation for effector caspases

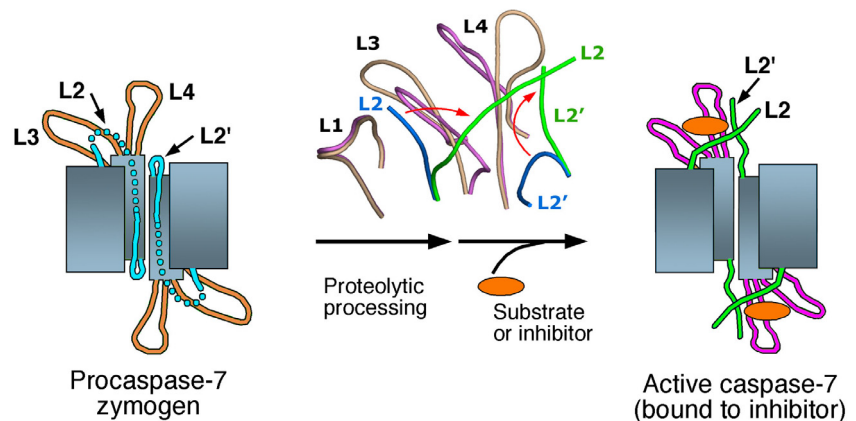
Comparison of the crystal structure of a representative effector caspase zymogen, procaspase-7, with that of the activated caspase-7 reveals a general molecular mechanism for the activation of effector caspases [24,25]. The essence of this mechanism is the formation of the activated conformation at the active site of caspase-7, which is conducive to substrate binding and catalysis (Figure 3). This conformation is critically supported by an open-ended surface loop, termed L2' loop, from the adjacent monomer and this loop becomes open-ended only after the activation cleavage in procaspase-7 (Figure 3), which allows the L2' loop to adopt the supporting configuration. In the procaspase-7 zymogen, the L2' loop is an internal loop and cannot adopt the supporting configuration. Consequently, the active site conformation does not support substrate binding or catalysis, thereby explaining why the procaspase-7 zymogen does not possess detectable catalytic activity. These observations also explain why effector caspases must remain as homodimers – because the active site conformation of one monomer relies on the indispensable support of the adjacent monomer.

It is worth noting that specific interactions between the L2'

loop and the active site are the indispensable requirement for the activation of an effector caspase. The activation cleavage merely allows these interactions to take place. When such interactions were ablated through partial deletion of the L2' loop, the resulting two-chain caspase-7 lost most of its catalytic activity [24]. Because the open end of the L2' loop is the amino-terminus of the small subunit, inverting the order of the large and small subunits at the primary sequence level is predicted to bypass the requirement of activation cleavage for an effector caspase. This prediction was confirmed by earlier studies on mammalian caspase-3 and caspase-6 [26] as well as the *Drosophila* caspase, Drice [27].

In addition to the essential role of the L2' loop, the activation cleavage allows for the appropriate placement of the active site loop L3, which is flexible in the zymogen but is well ordered in the active enzyme. In the active caspase-7, the amino-terminal region of the L3 loop extends into a cavity between the two caspase monomers; yet this space is partly occupied by the intra-chain linker in the procaspase zymogen. Thus it is possible that steric hindrance contributes to the inactive conformation of the L3 loop and the lack of catalytic activity [25].

Figure 3: Mechanism of activation for effector caspases as exemplified by caspase-7 [24,25]. A schematic diagram of procaspase-7 activation is shown here. The active-site loops before and after the proteolytic processing are shown in orange/cyan and magenta/green, respectively. The detailed conformational changes at the active site are depicted in the middle panel, in which the four surface loops, L1-L4, and the L2' loop are labeled. This figure was prepared partly using MOLSCRIPT [93].



Adaptor protein complexes for initiator caspase activation

The activation of an effector caspase is considered to be a direct consequence of the activation of a specific initiator caspase and does not involve any other co-factor. Consequently, the regulation for effector caspase activation is relatively simple and reasonably well understood. In contrast, the activation of an initiator caspase and its regulation are quite complex. All known initiator caspases undergo auto-catalytic intra-chain cleavage; yet compared to the effector caspases, this cleavage appears to have only modest effect on its catalytic activity and may not be required for their activation [28,29]. For example, the fully processed caspase-9 in isolation exhibits a low level of catalytic activity, similar to that of the unprocessed caspase-9 zymogen.

How is an initiator caspase activated then? The answer thus far relies on a specific adaptor protein complex. The activation of caspase-2, caspase-8, and caspase-9 in mammalian cells depends on the PIDDosome [30], the death-inducing signaling complex (DISC) [31], and the apoptosome [32-36], respectively (Figure 4). The primary component of the apoptosome is the apoptotic protease-activating factor 1 (Apaf-1). In the fruit fly *Drosophila*, the activation of Dronc (homolog of caspase-9) requires a protein complex involving Dark [37] (also known as Hac-1 [38] or Dapaf-1 [39], a homolog of Apaf-1). In the nematode *C. elegans*, the activation of CED-3 caspase zymogen is facilitated by CED-4 [40-45], which exhibits significant sequence homology to Apaf-1. In each case, the initiator caspase is recruited into and activated within the adaptor protein complex.

PIDDosome for caspase-2 activation

Caspase-2 is the second caspase identified in mammalian cells [46,47] and is required in stress-induced apoptosis [48]. It contains all hallmarks of an initiator caspase, including a caspase-recruitment domain (CARD) at its amino-terminus. The crystal structure of the CARD-deleted, activated caspase-2 revealed a disulfide bond at the dimeric interface [49], suggesting a critical role for dimerization. Subsequent characterization of caspase-2 activation *in vitro* showed that the auto-catalytic intra-chain cleavage significantly stabilizes dimer formation and drastically enhances the catalytic activity of caspase-2 [50]. However, the disul-

fide bond observed in the crystal structure appears to be dispensable for caspase-2 dimerization, as mutation of the relevant Cys residue at the dimeric interface did not affect the ability of recombinant caspase-2 to dimerize or to undergo auto-catalytic cleavage [50].

The activation of caspase-2 in cells requires the assembly of a large protein complex with a molecular weight in excess of 670-kDa [30,51]. Characterization of this complex revealed the presence of an adaptor protein RAIDD and the p53-induced protein with a death domain (PIDD) [30]. Subsequently the caspase-2-activating complex has been referred to as the PIDDosome [30] (Figure 4). In addition to the structure of caspase-2, the only other piece of structural information relevant to the PIDDosome is on the CARD domain of RAIDD [52], which is required for binding to the CARD domain of caspase-2. At present, the stoichiometry among PIDD, RAIDD, and caspase-2 remains enigmatic. It is also unclear whether any additional cofactor is involved in the PIDDosome. Most importantly, the molecular mechanism by which the PIDDosome facilitates the activation of caspase-2 remains unknown. Does PIDDosome simply promote the auto-catalytic cleavage of caspase-2 zymogen? Or can PIDDosome allosterically regulate caspase-2 activity in a way similar to the case of apoptosome to caspase-9 (see later)? The answer to the latter question is likely to be negative, because, in contrast to caspase-9, caspase-2 contains a caspase cleavage site between its amino-terminal CARD and the caspase unit. Nonetheless, definitive answers to these questions await experimental evidence.

DISC for caspase-8 activation

The extrinsic apoptosis pathway is triggered by the binding of a specific death ligand, such as FasL (also known as CD95L), to a specific transmembrane death receptor, such as Fas (also known as APO-1/CD95) [6]. The death receptors belong to the tumor necrosis factor (TNF) family, which contains a single death domain (DD) in the intracellular compartment [7]. Interestingly, the discovery of the DISC preceded and contributed to the identification of caspase-8. A large protein complex involving the activated Fas death receptor was found to assemble in the dying cells and named DISC [31]. One critical component of DISC was found to be Fas-associated death domain (FADD, also known as MORT1) [31,53], which was previously shown to interact with Fas

[54,55]. Another critical component of DISC was identified to be caspase-8 (also known as FLICE/MACH) [56,57]. Although the DISC contains additional cofactors and regulatory proteins such as FLIPs, Fas, FADD, and caspase-8 are the three essential and indispensable components [6] (Figure 4).

The function of DISC is to activate the initiator caspase, caspase-8, and perhaps caspase-10 as well [6]. The activated death ligands are homo-trimeric and thus induce oligomerization of the death receptors upon binding [58-60]. The receptor-associated adapter protein FADD contains a death effector domain (DED) at its amino-terminus and a DD at its carboxy-terminus; whereas caspase-8 has two copies of DED at its amino-terminus. FADD is thought to interact with Fas and the procaspase-8 zymogen through homotypic interactions, thus bringing three molecules of procaspase-8 into close proximity of one another and facilitating their auto-activation. The protein components of DISC are technically difficult to deal with; consequently, the activation of caspase-8 by DISC has yet to be reconstituted *in vitro* using recombinant proteins. Nonetheless, structures of the Fas DD [61], FADD DD [62,63], and FADD DED [64] have greatly facilitated identification of the interaction interfaces among components of the DISC [65,66].

At present, our understanding on the molecular mechanism of caspase-8 activation is derived almost entirely from studies on the isolated caspase-8. A prodomain-deleted uncleavable caspase-8 was found to exist mainly as a monomer by gel filtration; yet the catalytic activity predominantly associated with the fractions that corresponded to dimers [67,68]. In contrast, the wild-type (WT) caspase-8 exists in an equilibrium between monomers and dimers, with a dissociation constant of approximately 50 μ M, and binding to inhibitors results in enhanced dimerization for the WT caspase-8 [67]. These findings strongly argue that dimerization is a crucial factor for caspase-8 activation and suggest that DISC may facilitate the activation of caspase-8 through dimerization. This conclusion is further buttressed by inves-

tigation on how c-FLIP_L may activate caspase-8 [69-71].

Apoptosome for caspase-9 activation

Discovery of apoptosome

Caspase-9 is the most thoroughly characterized initiator caspase and its activation is mediated by the apoptosome, a multimeric complex involving Apaf-1, cytochrome *c*, and the cofactor dATP/ATP. Caspase-9 is the only initiator caspase whose activation by the adaptor complex has been successfully reconstituted *in vitro* using homogeneous recombinant proteins.

Cytochrome *c*, a crucial molecule for energy production in mitochondria, was identified as an important cellular activator of caspase-3 nine years ago [72]. Soon afterwards, the cellular receptor for cytochrome *c* was identified to be a novel protein, named Apaf-1 [73]. The oncoprotein Bcl-2 was shown to block apoptosis at least in part by preventing the release of cytochrome *c* from the mitochondria [74]. These observations support the concept that cytochrome *c* plays an essential role in apoptosis. But how does cytochrome *c* activate caspase-3? The answer was revealed by the identification of cytochrome *c* and dATP-dependent formation of an Apaf-1/caspase-9 complex [75], which subsequently activates the effector caspases, caspase-3 and -7.

Subsequent characterization revealed that, in the presence of dATP or ATP, cytochrome *c* and Apaf-1 assembles into a ~1.4 MDa complex, dubbed the "apoptosome" [32-36] (Figure 4). The CARD domain of Apaf-1 in the apoptosome interacts with the prodomain (also a CARD domain) of procaspase-9, resulting in the recruitment and subsequent activation of procaspase-9 zymogen [75,76]. Surprisingly, caspase-9 bound to the apoptosome exhibits a catalytic activity that is three orders of magnitude higher than that of the isolated active enzyme, prompting the concept of a holoenzyme [35]. The primary function of the apoptosome appears to be an allosteric regulation of caspase-9 activity. Supporting this notion, procaspase-9 zymogen possesses a

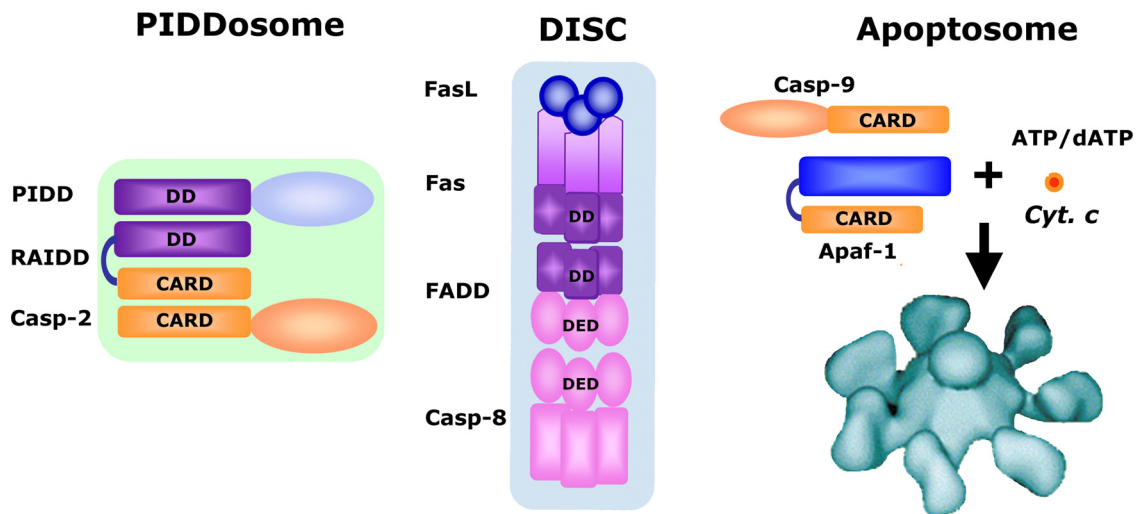


Figure 4: Adaptor protein complexes that are responsible for the activation of initiator caspases. The activation of caspase-2, caspase-8, and caspase-9 in mammalian cells are mediated by the PIDDosome [30], the death-inducing signaling complex (DISC) [31], and the apoptosome [32-36], respectively. PIDDosome contains at least three components, PIDD, RAIDD, and caspase-2 [30]. DISC is assembled following binding of death ligand to its receptor and contains FADD and caspase-8 (or -10). Apoptosome is composed of seven molecules of Apaf-1 bound to cytochrome *c* in the presence of ATP/dATP.

basal level of activity in the absence of the activation cleavage [28] and this activity can be up-regulated to the same level as the cleaved caspase-9 by the apoptosome [29].

Structure of Apaf-1 and the apoptosome

Apaf-1, the central component of the apoptosome, has three distinct domains, an N-terminal CARD, an expanded nucleotide-binding domain, and 12-13 WD40 repeats at its carboxy-terminal half. The CARD is responsible for interactions with the prodomain of caspase-9; this interaction is essential to the recruitment and activation of procaspase-9 [75,76]. The CARD and the nucleotide-binding domains are responsible for the oligomerization of Apaf-1 in the presence of cytochrome *c* and dATP; whereas the WD-40 repeats are thought to interact with cytochrome *c* as removal of this domain in Apaf-1 results in constitutive binding and activation of caspase-9 [77,78]. There are at least four distinct Apaf-1 splicing variants; but they all contain these three essential domains.

Apaf-1 exists in an inactive conformation in cells and is activated through binding to cytochrome *c* and dATP [75]. The structure of a WD40-deleted Apaf-1 revealed the underlying mechanism by which Apaf-1 maintains itself in an inactive state prior to dATP/ATP binding and explained why Apaf-1 needs the nucleotide for activation [79]. In the structure, five distinct domains, CARD, three-layered α/β domain, helical domain I, winged-helix domain, and helical domain II, pack closely against each other through extensive inter-domain interactions [79]. These interactions result in the burial of the caspase-9-binding interface. Unexpectedly, the bound nucleotide is ADP, which is deeply buried and serves as an organizing center to strengthen interactions among these four adjoining domains [79]. Structural analysis suggests that binding of nucleotide may induce significant conformational changes in Apaf-1 and that these conformational changes may drive the formation of the caspase-9-activating apoptosome.

How does the activated Apaf-1 assemble into an apoptosome? Structure of the apoptosome, determined at 27 Å resolution by cryo-electron microscopy (cryo-EM), reveals a wheel-shaped complex with seven-fold symmetry [80] (Figure 4). The CARD and the expanded nucleotide-binding domain of Apaf-1 are located at the central hub whereas the WD40 repeats constitute the extended spokes. Importantly, this structure confirms the structural involvement of cytochrome *c* in the formation of the apoptosome. Docking of caspase-9 to this apoptosome resulted in a dome-shaped structure in the center; however, the bulk of caspase-9 was not visible in these cryo-EM studies [80]. Limited by the low resolution, the apoptosome structure does not allow accurate identification of domain boundaries and interfaces. Consequently, the molecular underpinnings of caspase-9 activation remain to be identified.

Models of initiator caspase activation

Induced proximity model

Although the activation mechanism of effector caspases has been documented at molecular detail, how initiator caspases are activated remains inconclusive. The induced proximity model states that the initiator caspases auto-process themselves when brought into close proximity of each other [23]. This model elegantly summarizes experimental observations made by four independent laboratories, led by Dixit and Salvesen [81], Yang and Baltimore [45,82], Alnemri [77], and Spenser [83]. All four laboratories used a similar experimental approach, in which the target caspases were fused with a heterologous dimerization domain. The Alnemri group used the mouse IgG-Fc portion while the other three laboratories all employed tandem FK506-binding proteins (FKBPs), which bind to the dimeric ligand FK1012 and hence bring together the tethered caspases (Figure 5A). In all cases, the target caspases were processed upon induced oligomerization.

There is no doubt that induced proximity through the use of heterologous dimerization domains can lead to caspase activation. But this approach may not accurately recapitulate how the initiator caspases are activated under physiological conditions. The caspases used in these experiments were non-native hybrid proteins and undergo non-specific oligomerization. It is worth noting that all caspases, not only initiator but also effector caspases, undergo efficient autocatalytic cleavage when overexpressed in bacteria, presumably due to increased local concentrations or induced proximity. The use of the heterologous dimerization domains serves the purpose of increasing the local concentrations of caspases, thus allowing them to be auto-activated at considerably lower expression levels in mammalian cells. In this regard, the experiments performed in mammalian cells are analogous to bacterial overexpression; and caution must be exercised for the interpretation of these experiments. For example, although an effector caspase is activated specifically by an initiator caspase under physiological conditions, all known effector caspases can be efficiently auto-activated during bacterial over-expression. Caspase-3, a representative effector caspase, can also be auto-activated through the use of FKBPs in mammalian cells [83].

The initiator caspases are brought into close proximity of one another once they are recruited into the relevant adaptor protein complexes. Then these initiator caspases are somehow auto-activated. In this regard, the induced proximity model faithfully summarizes the process of initiator caspase activation at a general level but does not explain how the initiator caspases are activated at a mechanistic level. It is also worth noting that, at the time of the proposed induced proximity model, our understanding on the activation of initiator caspases was rather incomplete. For example, we did not understand that the activation of an initiator caspase, such as caspase-9, has little to do with the intra-chain cleav-

age [28,29]. The discovery that caspase-9 is activated only in the presence of the apoptosome as a holo-enzyme transformed our definition for the activation of initiator caspases [35].

Proximity-driven dimerization model

Caspases were generally thought to be homo-dimers. Thus, it came as a surprise that both procaspase-9 zymogen and the processed caspase-9 mainly exist as a monomer [84,85]. Using gel filtration, the processed caspase-9 monomers were separated from the dimers by gel filtration and were found to be catalytically inactive [84]. In contrast, the dimer fractions were highly active [84]. Structural analysis revealed that only one active site of the dimeric caspase-9 is functional and the other active site is unraveled and cannot support substrate binding [84]. Based on these findings, it was proposed that dimer formation may drive the activation of caspase-9 [84] (Figure 5).

Similar observations were reported for caspase-8. An uncleavable procaspase-8 zymogen was found to be predominantly monomeric in solution; however, the catalytic activity was exclusively associated with the gel filtration fractions that corresponded to dimers [67,68]. The processed caspase-8, on the other hand, exhibited a greater tendency to form dimers [67,68]. Gel filtration fractions that corresponded to the processed caspase-8 dimer exhibited a higher level of catalytic activity compared to those of the monomeric caspase-8 [67,68]. The monomers and dimers of the processed caspase-8 were found to be in equilibrium with a dissociation constant of approximately 50 μM , explaining why significant activity was detected in gel filtration fractions that corresponded to the monomers [67]. These observations suggest that dimer formation may drive the activation of caspase-8 [67,68].

The *in vitro* studies on caspase-9 and caspase-8 constitute a significant advance on our understanding of the mechanisms of activation for initiator caspases. The resulting hypothesis, proximity-induced dimerization [86] (Figure 5B), originates from but represents a qualitative advance over the induced proximity model, as it offers a mechanistic explanation for the activation of initiator caspases. This model may accurately reflect the mechanism of activation for some initiator caspases, such as caspase-8 [67,68] and caspase-2 [50], although this conclusion must be validated by studying how these initiator caspases are activated in their respective adaptor protein complexes.

Some of the critical supporting evidence for the proximity-induced dimerization hypothesis needs to be clarified. For example, gel filtration fractions that corresponded to the monomers of processed caspase-8 exhibited robust activity in one study [67] but was largely inactive in another study [68]. It is also perplexing how various caspases could be separated by their apparent molecular weights on native polyacrylamide gel electrophoresis [68], which is designed to separate native proteins by their charges and shape [87]. In addition, although dimers of the WT caspase-9 were present as a peak by gel filtration in one study [84], they were absent in another study using the same assay [88]. Furthermore, analysis by analytical ultracentrifugation suggested that caspase-9 existed exclusively as a monomer in solution [85].

Based on the proximity-driven dimerization model, the heptameric apoptosome can recruit multiple molecules of inactive procaspase-9 into close proximity of one another. The high local concentrations of procaspase-9 monomers in the apoptosome would favor dimerization and hence activation [80,84]. This explanation contains a strong assumption – the enzymatic activity of the dimeric caspase-9 should be identical to that of the apoptosome-bound caspase-9. To validate this assumption, the activity of the dimerized WT caspase-9 must be compared with that of the apoptosome-activated caspase-9. Unfortunately, WT caspase-9 homo-dimer is transient in solution and cannot be isolated. To circumvent this problem, one approach is to engineer a constitutively dimeric caspase-9 – this was achieved by replacing five residues at the dimerization interface with those from caspase-3 [88]. Structure of the engineered dimeric caspase-9 was found to closely resemble that of the WT caspase-9, including all relevant structural details [88]. Compared to the WT caspase-9, this engineered dimer exhibits a higher level of catalytic activity *in vitro* and induces more efficient cell death when expressed [88]. However, the catalytic activity of the dimeric caspase-9 is only a small fraction of that for the Apaf-1-activated caspase-9 [88]. This finding suggests that dimerization of caspase-9 may be qualitatively different from its activation by the apoptosome [88].

Induced conformation model

The fact that caspase-9 exhibits a much higher level of catalytic activity in the apoptosome demonstrates that a conformational change must have occurred in the active site of the apoptosome-bound caspase-9, resulting in higher K_m and/or K_{cat} for the substrates. The proximity-driven dimerization model argues that this conformational change is identical to that seen in the monomer-to-dimer transition of an initiator caspase.

There are additional possibilities. One model is that the apoptosome may directly activate monomeric caspase-9 through modification of its active site conformation [85] (Figure 5C). Under this scenario, one way the apoptosome may activate the monomeric caspase-9 is to bind to its surface that is required for its homo-dimerization, thus stabilizing the productive conformation of the active site. This strategy is similar to the way by which the L2' loop stabilizes the active site of an effector caspase (Figure 3). Consistent with this model, the BIR3 domain of XIAP specifically hetero-dimerizes with caspase-9 through an interface that is also required for the homo-dimerization of caspase-9 [85]. This model could also explain the observed phenotype of the interface mutant caspase-9 [68].

An alternative model is that the apoptosome assembles the dimeric caspase-9 into a higher-order complex (Figure 5C), which results in the modification of the active site conformation for an enhanced activity [89]. Consistent with this hypothesis, in the crystals of the inhibitor-bound caspase-9, caspase-9 exists as a dimer of homo-dimer, or a homo-tetramer [84]. The specific interactions between the two homo-dimers are more extensive than regular crystal packing contacts and directly affect the conformation of the active sites [84]. A third possibility is that the apoptosome forces the WT monomeric caspase-9 to dimerize (Figure 5C);

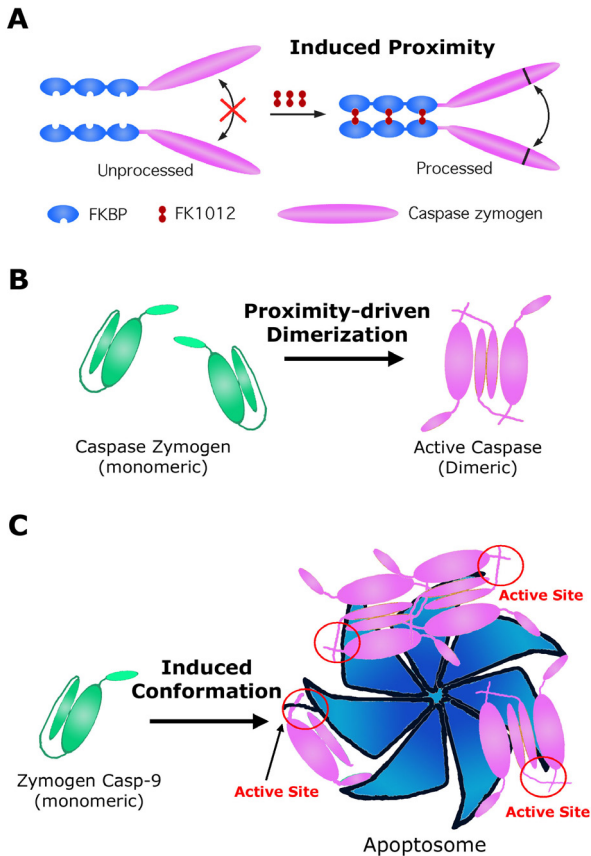


Figure 5: Models of initiator caspase activation. (A) Induced-proximity model [23]. The induced proximity model states that the initiator caspases auto-process themselves when brought into close proximity of each other [23]. In the primary supporting experiments, the target caspases were fused with a heterologous dimerization domain, such as tandem FK506-binding proteins (FKBPs) shown here. Binding to the dimeric ligand FK1012 brings together the tethered caspases, resulting in their activation. (B) Proximity-driven dimerization model [86]. This model states that dimer formation drives the activation of initiator caspases and the adaptor protein complexes serve to promote dimerization by increasing the local concentrations of initiator caspases. (C) Induced conformation model [89,91]. This model states that the activated conformation of the active site for a given initiator caspase is the reason for its activation and is attained through direct interaction with the adaptor protein complex or through homo-oligomerization facilitated by the adaptor protein complex. Three scenarios are depicted here.

but this process must involve not just increased local concentration but also additional interface between the apoptosome and caspase-9 [90](see later). In this scenario, it is also possible that the dimerized caspase-9 in the context of the apoptosome exhibits a perturbed interface relative to the crystallographically observed dimer interface, which may greatly facilitate the catalytic activity of caspase-9.

These hypotheses are collectively termed “induced conformation” model (Figure 5C), in which conformational changes at the active site are regarded as the ultimate reason for caspase activation [89]. Although available data are insufficient to differentiate among various specific models, exquisite conformational changes of caspase-9 must be induced upon binding to the apoptosome [90]. Previous biochemical studies revealed that the CARD domain of Apaf-1 oligomerizes caspase-9 into a multimeric complex, with a molecular weight of approximately 300-kDa [90]. Because Apaf-1 CARD only forms a stable hetero-dimer with the prodomain of caspase-9 [75,76], the formation of this large “CARD/caspase-9” complex must involve an addi-

tional interface between the catalytic subunit of caspase-9 and Apaf-1 CARD. This complex was thought to represent the strapped-down version of the apoptosome as caspase-9 within this complex exhibited significantly elevated catalytic activity compared to the isolated caspase-9 [90]. Regardless of the catalytic activity, the formation of the 300-kDa complex between Apaf-1 CARD and caspase-9 demonstrates that other interactions, in addition to those between their respective CARD domains, exist between caspase-9 and Apaf-1. This interaction may play an important role in the activation of caspase-9 within the apoptosome.

Conclusion and perspective

Despite intense investigation, we have only studied the mechanism of activation for a few initiator caspases, mostly relying on isolated proteins without the involvement of the adaptor protein complexes that are responsible for their activation in cells. Nonetheless, we have begun to gain important insights into this process. The induced proximity model summarizes the general process of initiator caspase activation [23]. The proximity-driven dimerization model describes how initiator caspases respond to induced proximity and offers a specific explanation to how the initiator caspases are activated [86]. No matter how caspases are activated, enhanced catalytic activity must be correlated with altered active site conformation. In this regard, the induced conformation model was proposed to explain the activation of initiator caspase activation [89,91]. Both proximity-driven dimerization and induced conformation hypotheses are extensions of the induced proximity model.

The induced conformation model is different from the proximity-driven dimerization model in that different aspects are emphasized for the activation of initiator caspases – the former stresses the importance of active site conformation whereas the latter highlights the dimerization process. The induced conformation model states that the activated conformation of the active site for a given initiator caspase is attained through direct interaction with the adaptor protein complex or through homo-oligomerization facilitated by the adaptor protein complex. But these two models are not always mutually exclusive. For example, the essence of proximity-driven dimerization for an initiator caspase is to orient the active site conformation for more efficient substrate binding and catalysis [17]. For some initiator caspase(s), dimerization might be sufficient for inducing the correct conformation that is needed for its activation. In this case, these two models are in agreement with each other. However, for some other caspase(s), such as caspase-9, dimerization itself is unlikely to be the sole mechanism for its activation [88].

Although the activation of effector caspases employs significantly different molecular mechanisms [14], it also requires the formation of an activated conformation at the active site (Figure 3). The effector caspase zymogens are constitutively dimeric in solution; but their active sites exist in an inhibited conformation [24,25]. The intra-chain cleavage, mediated by a specific initiator caspase, allows a critical loop in one caspase monomer to change conformation and to stabilize the active site loops in the adjacent monomer [24,25]. In this regard, both effector and initiator caspases share the same basic mechanism for their catalytic

activation – attaining an activated conformation at the active site, although the means by which they achieve this conformation are different. While effector caspases only require a specific intra-chain cleavage, the initiator caspases rely on adaptor protein complexes. The underlying molecular mechanisms by which the adaptor protein complexes activate initiator caspases remain to be elucidated by biochemistry, biophysics, and structural biology.

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