Caspase Activation: Revisiting the Induced Proximity Model

Minireview

Yigong Shi*
Department of Molecular Biology
Princeton University
Princeton, New Jersey 08544

Caspases execute cell death. The mechanism of effector caspase activation primarily involves reorganization of active site loops following the activation cleavage. The Induced Proximity hypothesis, originally proposed to explain the activation of initiator caspases, has recently been reinterpreted to be proximity-driven dimerization of initiator caspases, and consequently their activation. The evidence supporting these models is critically evaluated and other possible mechanisms for initiator caspase activation are discussed.

The molecular hallmark of programmed cell death, or apoptosis, is the activation of caspases (Thornberry and Lazebnik, 1998). Caspases are synthesized as relatively inactive zymogens and must undergo a process of activation during apoptosis. Based on their order of activation, caspases are classified into two families: the initiator caspases and the effector caspases (Shi, 2002). In response to upstream apoptotic stimuli, the initiator caspases (also known as "apical caspases") undergo a complex course of autocatalytic processing and activation, which usually require several auxiliary factors (Shi, 2002). Once activated, an initiator caspase specifically cleaves and hence activates an effector caspase zymogen. For example, the initiator caspase-9 is activated by the assembly of a multimeric complex (dubbed apoptosome) involving Apaf-1 and cytochrome c. Once activated, caspase-9 cleaves and activates caspase-3 and caspase-7. Known for their rapid catalytic turnover, caspases-3 and -7 degrade a large number of cellular proteins that ultimately kill a cell (Thornberry and Lazebnik, 1998).

The Definition of Caspase Activation

Caspases are synthesized as a single-chain zymogen. The activation of an effector caspase zymogen is defined as the intrachain cleavage mediated by a specific initiator caspase. As a consequence of the intrachain cleavage, the catalytic activity of an effector caspase is enhanced by several orders of magnitude.

For the initiator caspases, however, the definition of activation is quite different. An initiator caspase undergoes an autocatalytic intrachain cleavage; yet compared to the effector caspases, this cleavage appears to have only modest effect on its catalytic activity (Srinivasula et al., 2001; Stennicke et al., 1999). For example, the fully processed caspase-9 in isolation is only marginally active, much the same way as the unprocessed caspase-9 zymogen. In sharp contrast, association with the apoptosome leads to a dramatic increase (up to 2000-fold) in the catalytic activity of the processed as well as

*Correspondence: yshi@molbio.princeton.edu

the unprocessed caspase-9 (Rodriguez and Lazebnik, 1999; Srinivasula et al., 2001). Thus, for caspase-9, activation has little to do with the intrachain cleavage; rather, it refers to the apoptosome-mediated enhancement of the catalytic activity of caspase-9. In this regard, the use of procaspase-9 processing as an indicator for its activation may not reflect the physiological settings in cells.

How about other initiator caspases? The answer is that we simply don't know. Most biochemical studies on initiator caspases have been focused on caspase-9. It remains to be seen whether the definition of caspase-9 activation is truly representative among the other initiator caspases. Caspase-8 is another initiator caspase whose activation has been biochemically analyzed in vitro; yet this has not been done in the context of its physiological activation complex, the Death-Inducing Signaling Complex (DISC).

Mechanism of Activation for the Effector Caspases

Based on biochemical and structural characterization, an effector caspase is known to exist constitutively as a homo-dimer, both before and after the intra-chain activation cleavage (Shi, 2002). The mechanism of activation for a representative effector caspase, caspase-7, is manifest from the conformational change of the active site following the activation cleavage.

The "activated" active site conformation is revealed by the structure of the processed caspase-7 bound to a covalent peptide inhibitor (Wei et al., 2000) (Figure 1A). The active site comprises four surface loops, L1 through L4, all from the same monomer (Figure 1A). L1 and L4 constitute two sides of the substrate binding groove; L3 forms the base (Figure 1B). The catalytic cysteine resides in loop L2, poised for catalysis (Figure 1B). Importantly, the L2′ loop, which comes from the adjacent monomer, plays a significant role in stabilizing the "activated" conformation of the active site through intimate interactions with loops L2 and L4. It is of interest to note that the backbone configuration of active site, including the L2′ loop, is highly similar for all inhibitor bound caspases (Shi, 2002; Figure 1B).

The "inhibited" active site conformation before the intrachain cleavage is revealed by the crystal structure of the unprocessed procaspase-7 zymogen (Chai et al., 2001; Riedl et al., 2001). Compared to the processed and inhibitor bound caspase-7, the L2′ loop is flipped by 180 degrees (Figure 1C), which is necessitated by the uncleaved peptide linkage. The conformational switch of the L2′ loop results in disruption of the interactions it makes with loops L2 and L4, which in turn leads to an inhibited conformation of the active site (Figure 1C). Compared to the activated conformation, Loop L2 is also twisted, making the catalytic cysteine inaccessible to solvent. Loops L3 and L4 also undergo some conformational changes; however, their conformations are less well defined as judged by their relatively high temperature factors (Chai et al., 2001; Riedl et al., 2001), which correlate with a high degree of flexibility.

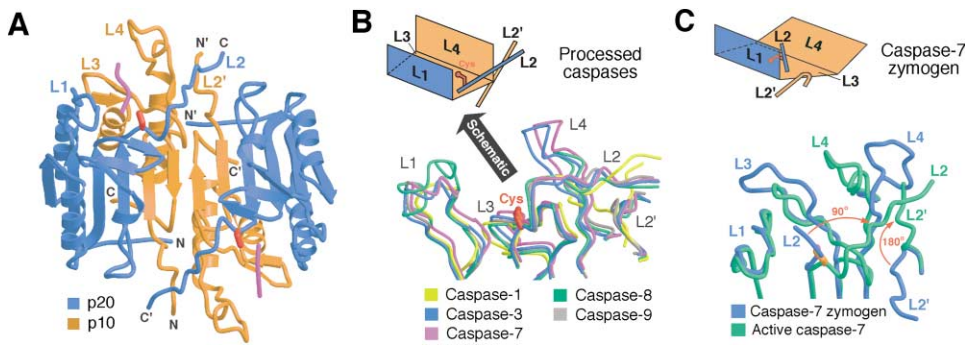If the L2′ loop is indeed the critical determinant for

Figure 1. Molecular Mechanism for the Activation of Caspase-7, a Representative Effector Caspase

(A) A schematic diagram of the active caspase-7 bound to a covalent peptide inhibitor. The p20 and p10 subunits are colored blue and orange, respectively. The catalytic cysteine and the bound inhibitor are highlighted in red and magenta, respectively. The four active site loops (L1–L4) and the critical supporting loop (L2′) from the adjacent monomer are labeled.

(B) The backbone configuration of the active site is highly conserved among all active caspases (lower panel). The active sites from five representative caspases are color-coded as shown. The substrate binding groove is schematized and shown above.

(C) The active site conformation in the procaspase-7 zymogen is dramatically different from that of the active caspase-7. Compared to the active caspase-7 (green), the L2′ loop in the procaspase-7 zymogen (blue) is flipped by 180 degrees, because it is covalently linked to its N-terminal sequences in the absence of proteolytic processing. The loss of support by the L2′ loop results in the unraveling of the active site conformation, which is no longer conducive to catalysis.

the activation of an effector caspase, then removing it should render the resulting caspase largely inactive. Indeed, this has been shown true for caspases-3 and -7 (Chai et al., 2001).

The Induced Proximity Model

The Induced Proximity model, first proposed in 1998, states that the initiator caspases autoprocess themselves when brought into close proximity of each other (Salvesen and Dixit, 1999). This model is a succinct summary of experimental observations made by four independent laboratories, led by Dixit and Salvesen (Muzio et al., 1998), Yang and Baltimore (Yang et al., 1998a, 1998b), Alnemri (Srinivasula et al., 1998), and Spenser (MacCorkle et al., 1998). The experimental approach was similar for all four groups—in each case the target caspase was fused with a heterologous dimerization domain (Figure 2). The Alnemri group used the mouse IgG-Fc portion while the other three laboratories all employed tandem FK506 binding domains (FKBPs), which bind to the dimeric ligand FK1012 and hence bring together the tethered caspases. As all four groups demonstrated, the target caspases were processed upon induced oligomerization.
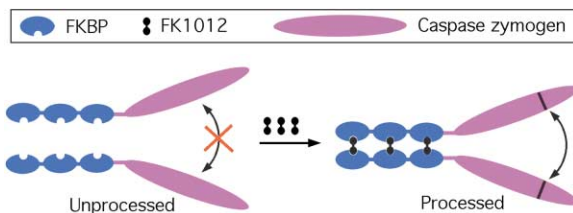

Figure 2. Schematic Diagram of the Key Experiment Supporting the Induced Proximity Model

Caspases (magenta) are fused with heterologous dimerization motifs such as the FK506 binding domain (FKBP, shown in blue). Incubation of the dimerizing ligand, such as FK1012 (shown as a black dumbbell), with FKBP, results in its dimerization and hence induced proximity of the tethered caspase molecules. Caspase processing ensues.

At the time when the Induced Proximity model was introduced, our understanding of the initiator caspases was rather incomplete. For example, the activation of an initiator caspase was generally thought to be mediated by the intrachain cleavage, much the same way as the effector caspases. Only later was it shown definitively that the activation of an initiator caspase, such as caspase-9, may not be linked to the intrachain cleavage (Stennicke et al., 1999). The discovery that caspase-9 is activated only in the presence of the apoptosome as a holoenzyme transformed our definition for the activation of initiator caspases (Rodriguez and Lazebnik, 1999). Consequently, the Induced Proximity hypothesis may have to be reevaluated.

Although induced proximity through the use of heterologous dimerization domains can lead to caspase activation, it may not be an accurate reflection of how the initiator caspases are activated under physiological conditions. The experimental observations supporting the Induced Proximity model were based on an artificially engineered system, in which the caspases were nonnative hybrid proteins and undergo nonspecific oligomerization. In this context it is worth noting that caspases are easily autoactivated when overexpressed in bacteria. The use of the heterologous dimerization domains significantly increases the local concentrations of caspases, thus allowing them to be autoactivated at considerably lower expression levels in mammalian cells. These experiments performed in mammalian cells are thus analogous to bacterial overexpression. Similar to the initiator caspases, effector caspases can also be autoactivated when overexpressed in bacteria, likely through Induced Proximity; yet in mammalian cells they are activated specifically by the initiator caspases. In addition, caspase-3, a representative effector caspase, can also be auto-activated through the use of FKBPs in mammalian cells (MacCorkle et al., 1998). Thus, the key experiments supporting the Induced Proximity model can be interpreted otherwise. More importantly, although it elegantly summarizes a number of observa-

tions, the larger issue is that at a mechanistic level, the Induced Proximity model does not explain how the initiator caspases are activated.

Dimerization-Driven Activation?

An essential feature of cellular biochemistry is the stringent specificity that guarantees the faithful execution of a biological process. However, the key experiments that led to the proposition of the Induced Proximity model did not factor in specificity—the specific protein-protein interactions that are required for the precise positioning and activation of the initiator caspases (MacCorkle et al., 1998; Muzio et al., 1998; Srinivasula et al., 1998; Yang et al., 1998a, 1998b). This shortcoming has been nicely remedied by Salvesen and colleagues, who proposed, through biochemical studies of caspases-9 and -8, that dimerization of the initiator caspases drives their activation (Boatright et al., 2003; Donepudi et al., 2003; Renatus et al., 2001). This hypothesis, termed proximity-induced dimerization (Boatright et al., 2003), represents a qualitative advance over the previous Induced Proximity model, as it takes into account the specific caspase conformation as the underpinnings of initiator caspase activation. It should be noted that, homodimerization of the caspase monomers, which strictly depends on their specific interfaces, is entirely different from the nonspecific oligomerization caused by the heterologous dimerization domains. In the latter case, caspases are merely brought close to each other and have never been shown to dimerize through their intrinsic dimerization interface.

One important piece of supporting evidence for the proximity-induced dimerization model was the observation that caspase-9 existed in an equilibrium between a major fraction of monomers and a minor fraction of homodimers as judged from a gel filtration analysis (Renatus et al., 2001). Interestingly, fractions corresponding to the homodimers from gel filtration exhibited a robust catalytic activity in an in vitro assay while the monomers were largely inactive (Renatus et al., 2001). However, it is unclear why the diluted homodimers of caspase-9 did not dissociate into the inactive monomers after gel filtration or why the more concentrated caspase-9 monomers did not form the active homodimers. One possible explanation is that the monomers and the homodimers of caspase-9 may undergo an extremely slow exchange, resulting in the kinetic trapping of caspase-9 homodimers. But this explanation is not supported by the structural observation, which shows the unfavorable packing interactions at the homodimeric interface of caspase-9 (Renatus et al., 2001). In contrast, analyses by analytical ultracentrifugation revealed that caspase-9 existed as a single species with a molecular weight consistent with that of a monomer (Shiozaki et al., 2003).

Based on the proximity-induced dimerization model, the function of the apoptosome is to promote the homodimerization of caspase-9 due to its increased local concentrations (Renatus et al., 2001). Similarly, the DISC is thought to induce the dimerization and subsequent autoactivation of caspase-8 (Boatright et al., 2003). These predictions are yet to be confirmed experimentally. Interestingly, the apoptosome has a 7-fold symmetry (Acehan et al., 2002) whereas the DISC originates from the homotrimeric assembly of the death ligand-receptor complexes. It is unclear how these macromo-

lecular assemblies with an odd number of symmetry promote the dimerization of bound caspases.

The 27 Å structure of the apoptosome reveals its general architecture but not the mechanism for the activation of caspase-9 (Acehan et al., 2002). In the absence of a high-resolution structure of the caspase-9/apoptosome complex, the only way to examine the dimerization-driven activation model is to perform robust biochemical analyses. If homodimerization is indeed the mechanism responsible for caspase-9 activation, then the activity of the homodimeric caspase-9 should be the same as that of caspase-9 bound to the apoptosome. Thus, a direct comparison of enzymatic activity in these two cases will provide a definitive answer to the validity of the dimerization-driven activation model. Unfortunately, the dimeric caspase-9 cannot be isolated, because caspase-9 exists as a stable monomer in solution (Shiozaki et al., 2003). Perhaps engineering of a homodimeric caspase-9 will reveal some useful information.

Induced Conformation Models

Biochemical and structural analyses revealed that the homodimerization of caspase-9 resulted in the formation of only one functional active site (Renatus et al., 2001) (Figure 3A). This information, in conjunction with the behavior of the apoptosome under cryoelectron microscope, prompted the proposition of a working model to explain the activation of procaspase-9 (Acehan et al., 2002) (Figure 3B, Scenario I). In this model, a heptameric apoptosome binds seven monomers of inactive caspase-9. The high local concentrations of caspase-9 within this apoptosome promote the efficient recruitment of additional inactive caspase-9 monomers, which become activated upon binding. This model is a refinement of the proximity-induced dimerization model. An explicit assumption of this model is that high local concentrations of caspase-9 monomers within the apoptosome should favor dimerization with additional monomers in solution. However, this assumption may not be valid as the increased local concentration is at the expense of a proportionally decreased concentration elsewhere. Alternatively, as is usually the case in biology, the high local concentrations of caspase-9 monomer within the apoptosome may drive the internal dimerization of caspase-9, hence resulting in its activation (Figure 3B, Scenario II).

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 (Shiozaki et al., 2002). 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 (Shiozaki et al., 2002). 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, must exist between caspase-9 and Apaf-1. This elusive interaction may play an important role in the activation of caspase-9 within the apoptosome.

Two additional, speculative models can be proposed to explain the activation of caspase-9, with neither contradicted by any published evidence (Figures 3C &
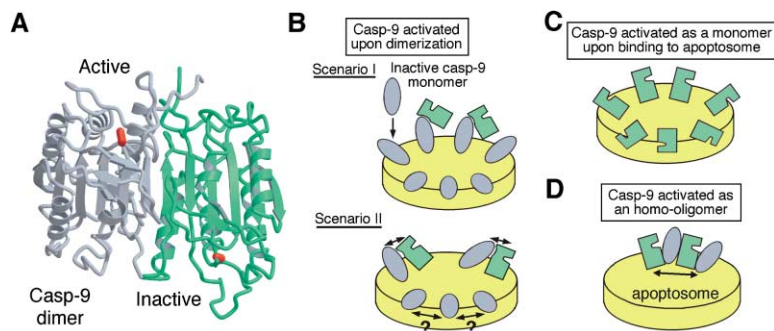
Figure 3. Models of Caspase-9 Activation by the Apoptosome

(A) Structure of the inhibitor-bound caspase-9 homo-dimer. Only one active site exists in a productive conformation in the homo-dimer. (B) Two scenarios of the proximity-induced dimerization model. In scenario I (upper image), the high local concentrations of inactive caspase-9 monomers are thought to favor the recruitment of additional inactive monomers, which become activated upon dimerization. In scenario II (lower image), the high local concentrations of inactive caspase-9 monomers within the apoptosome favor the dimerization among each other. Both scenarios rely on the unproven assumption that the catalytic activity of the caspase-9 homodimer is equivalent to that of caspase-9 in the apoptosome. (C and D) In these induced conformation models, caspase-9 undergoes a conformational change upon binding to the apoptosome. This change gives rise to the enhanced catalytic activity. The conformational change can be exerted to caspase-9 in its monomeric form (C) or an oligomeric form (D).

3D). Both models rely on additional interactions between Apaf-1 and caspase-9 and involve conformational changes in the active site, hence termed "Induced Conformation" models. In the first model (Figure 3C), caspase-9 is activated as a monomer upon its binding to the apoptosome. Under this scenario, one way the apoptosome may activate the monomeric caspase-9 is to bind to its surface that is required for its homodimerization and stabilizes the productive conformation of the active site, much the same way as the critical L2′ loop for effector caspases (Figure 1B). Consistent with this model, the BIR3 domain of XIAP specifically heterodimerizes with caspase-9 through an interface that is also required for the homodimerization of caspase-9 (Shiozaki et al., 2003). In the second model (Figure 3D), caspase-9 forms a homooligomer in the apoptosome, in which the productive conformation of the active site is stabilized (Figure 3D). Interestingly, in the crystals of inhibitor bound caspase-9, caspase-9 does not merely exist as a homodimer, but also exists as a dimer of homodimer, or a homo-tetramer (Renatus et al., 2001). The specific interactions between the two dimers are more extensive than normal crystal packing contacts and directly affect the conformation of the active sites (Renatus et al., 2001).

Perspective

The original description of the Induced Proximity model was a general summary of what had been observed experimentally in laboratories and does not reveal the molecular mechanisms for the activation of initiator caspases. The proposal of the proximity-induced dimerization model as the mechanistic explanation represents a refined version of the Induced Proximity model and addresses the specificity issue in caspase activation. Nonetheless, this model awaits further testing, as the critical experiments are yet to be performed. In addition, other models are also compatible with available evidence.

At present, our understanding on the activation of initiator caspases is very limited. We do not yet know the molecular mechanism for the activation of any initiator caspase by its upstream activation complex. Before we have a comprehensive list of mechanistic case studies on the initiator caspases, we will not be able to derive a set of general principles that govern the activation mechanisms of the initiator caspases.

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