Structures, Domains and Functions in Cell Death (DD, DED, CARD, PYD)

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The death domain (DD), death effector domain (DED), caspase-recruitment domain (CARD) and pyrin domain (PYD) are subfamilies of the DD superfamily. By mediating homotypic interactions, these proteins play important roles in the assembly and activation of apoptotic signalling complexes. They are responsible for caspase recruitment and for formation of oligomeric platforms for signalling. DD superfamily proteins have a common six-helical bundle fold and show different surface features for each of the subfamilies. Most interestingly, the homotypic interactions within each subfamily are mostly mediated by asymmetric contacts, in which different surfaces of the interaction partners are adjacent to each other. The DD superfamily proteins appear to use three common types of asymmetric interactions to assemble into large oligomeric complexes.

Introduction

Apoptosis is an orderly cellular suicide programme, that is, critical for the development and homeostasis of a multicellular organism. Failure to control apoptosis can lead to serious diseases that threaten the existence of the organism (Rathmell and Thompson, 2002). Apoptosis proceeds through characteristic morphological changes that are dependent on caspase activities. Caspasess are cysteine proteases that cleave specifically after aspartic acid residues (Riedl and Shi, 2004). Because caspasess are the executioners of apoptosis, they are the key players in apoptotic cell death.

On a molecular level, the death domain (DD) superfamily members are involved in formation of oligomeric signalling complexes for caspase activation. A central paradigm in caspase activation is the assembly of oligomeric signalling complexes in response to internal or external stimuli. In a simplified view, these molecular complexes activate their effectors via ‘proximity-induced autoactivation’ such as dimerization and proteolytic autoprocessing (Salvesen and Dixit, 1999). The DD superfamily plays a critical role in this assembly by participating in both self-association and other protein:protein interactions.

The DD superfamily is one of the largest and most studied domain superfamilies (McEntyre and Gibson, 2004). It is currently composed of four subfamilies, the DD, the death effector domain (DED), the caspase recruitment domain (CARD) and the pyrin domain (PYD) subfamilies (Reed et al., 2004). DDs, DEDs, CARDs and PYDs are involved in both caspase activation and the related process of nuclear factor-kB (NFkB) activation in host defence. The DD superfamily proteins are evolutionarily conserved in many multicellular organisms such as mammals, Drosophila and Caenorhabditis elegans. Based on a genome analysis, there are 32 DDs, 7 DEDs, 28 CARDs and 19 PYDs in the human genome (Reed et al., 2004).

Deregulation of caspase activation is related to many human diseases. Most notably, defective receptor-mediated caspase activation and cell death underlies the human genetic disease autoimmune lymphoproliferative syndrome (ALPS) (Poppema et al., 2004; Rieux-Laucat et al., 2003b). When lymphocytes from patients with ALPS are cultured in vitro, they are resistant to apoptosis as compared to cells from healthy controls. Most patients with ALPS have mutations in the intracellular DD of the receptor (Rieux-Laucat et al., 2003a).

Functions of DDs, DEDs, CARDs and PYDs: Assembly of Caspase-activating Signalling Complexes

DDs, DEDs, CARDs and PYDs participate in homotypic interactions within the same subfamily. Many proteins involved in apoptosis contain these domains (Figure 1). Some examples of these important signalling complexes are presented in the next section.
Death-inducing signalling complex for activation of caspase-8 and caspase-10 and its inhibition by FLIPs

Fas (also known as CD95) is a prototypical member of death receptors that form a subfamily of the tumour necrosis factor (TNF) receptor superfamily to mediate the extrinsic cell death pathway. Members of the TNF superfamily of ligands are mostly trimeric and activate the TNF receptor superfamily by ligand-induced receptor trimerization and higher order oligomerization (Kischkel et al., 1995). The intracellular regions of death receptors contain DDs (Tartaglia et al., 1993). For Fas, on ligand activation, its DD recruits the Fas-associated DD (FADD) adapter protein via a homotypic interaction with the C-terminal DD of FADD (Chinnaiyan et al., 1995; Kischkel et al., 1995). FADD also contains an N-terminal DED that interacts homotypically with the tandem DED in the prodomain of caspase-8 or -10 (Wajant, 2002). These interactions form the ternary death-inducing signalling complex (DISC)-containing Fas, FADD and caspase-8 or -10 (Wajant, 2002). Recruitment of pro-caspases into the DISC initiates caspase proteolytic auto-processing. This liberates active caspase-8 or -10 into the cytoplasm to cleave and activate effector caspases such as caspase-3 and caspase-7, leading to a cascade of events in apoptotic cell death.

Caspase activation by the DISC is inhibited by FLIPs, a family of cellular and viral tandem DED-containing proteins that interact with FADD (Thome and Tschopp, 2001). Cellular FLIPs (c-FLIPs), comprising the long and short isoforms, c-FLIP-L and c-FLIP-S, are tightly regulated in expression in T cells and may be involved in controlling both T-cell activation and death (Thome and Tschopp, 2001). v-FLIPs (viral FLIPs) appear to have evolved to inhibit apoptosis of virally infected host cells and are present in the poxvirus Molluscum contagiosum virus (MCV) as proteins MC159 and MC160 (Shisler and Moss, 2001) and in γ-herpesviruses (Thome and Tschopp, 2001).

Apoptosome for caspase-9 activation

The intrinsic pathway of apoptotic cell death is induced in a mitochondria-dependent manner in response to intracellular insults. A CARD-containing protein Apaf-1 (apoptosis-activating factor 1) forms the central platform of a molecular
complex known as the apoptosome for caspase activation in this pathway (Li et al., 1997). Apaf-1 is composed of an N-terminal CARD, a central nucleotide-binding oligomerization domain (NOD) and a C-terminal (Trp-Asp) WD repeat domain. On mitochondrial leakage, cytochrome c, which normally resides at the intermembrane space of the mitochondria, is released to the cytosol. The interaction of cytochrome c with the WD repeat domain of Apaf-1 presumably opens up the Apaf-1 structure, leading to an adenosine triphosphate (ATP)- or dATP-dependent oligomerization of Apaf-1 to form the apoptosome. The apoptosome then recruits caspase-9 via the CARD domain interaction between Apaf-1 and caspase-9.

**PIDDosome for caspase-2 activation**

In response to genotoxic stress, the DD-containing and p53-induced protein with a death domain (PIDD) mediates both caspase-2 and NFκB activation. Caspase-2 is an evolutionarily conserved initiator caspase with a CARD prodomain (Wang et al., 1994). The caspase-2 activation pathway involves the formation of a ternary complex known as the PIIDosome (Berube et al., 2005; Tinel and Tschopp, 2004), which comprises proteins PIDD (Lin et al., 2000), RAIDD (RIP-associated ICH-1 homologous protein with a death domain; Duan and Dixit, 1997) and caspase-2 and is assembled via the DD interaction between PIDD and RAIDD and the CARD interaction between RAIDD and caspase-2. Accumulating data have shown that caspase-2 acts upstream of the mitochondria to initiate apoptosis (Lassus et al., 2002).

**Inflammasome for caspase-1 activation**

Members of the Nod-like receptor (NLR) family, including NLRP1, NLRP3 and NLRC4, and the adaptor ASC (apoptosis-associated speck-like protein containing a CARD) are critical components of the inflammasome that link microbial and endogenous ‘danger’ signals to caspase-1 activation (Franchi et al., 2009). NLR family proteins are PYD- and NOD-containing proteins. For example, NLRP1 contains an N-terminal PYD, a central NOD for oligomerization, a leucine-rich repeat (LRR) region and a C-terminal CARD. ASC contains both a PYD and a CARD. Caspase-1 is recruited to the inflammasome either via ASC or directly to NLR proteins. Once activated, caspase-1 cleaves and activates the cytokine interleukin 1β (IL-1β), leading to recruitment of inflammatory cells to the site of infection and a specific form of cell death called ‘pyroptosis’. Direct linkage between pathogen invasion and inflammasome formation has been obscure. A series of recent studies provided advancement in this regard by showing that a PYD-containing protein AIM2 (absent in melanoma 2) senses cytoplasmic foreign double-standard deoxyribonucleic acid (dsDNA) and interacts with ASC for caspase-1 activation (Burckstummer et al., 2009; Fernandes-Alnemri et al., 2009; Hornung et al., 2009; Roberts et al., 2009).

**Structures of Isolated Domains and Their Surface Features**

The unifying feature of the DD superfamily is the six-helical bundle structural fold as exemplified by structures of Fas DD (Huang et al., 1996; Figure 2a), FADD DD (Eberstadt et al., 1998; Figure 2b), Apaf-1 CARD (Qin et al., 1999; Figure 2c) and NLRP1 PYD (Hiller et al., 2003; Figure 2d). Although all members of the DD superfamily have this conserved structural fold, individual subfamilies also exhibit distinct structural and sequence characteristics not shared with other subfamilies. There are currently structures of seven DDs, four DEDs, five CARDs and five PYDs. Because many DDs seem to self-associate and have a tendency to aggregate, their structures were often determined under nonphysiological conditions such as extreme pH and/or with ‘de-aggregating’ mutations (Huang et al., 1996). Of the four DED structures, three are more similar to each other than to other members of the DD superfamily. In contrast, the MC159 DED1, which was solved in the context of a tandem DED, is structurally more divergent from the other known DED structures (Li et al., 2006; Yang et al., 2005). In particular, helix H3 is missing and replaced by a short loop connecting helices H2 and H4. Two additional helices are present, helix H0 at the N-terminus and helix H7 that brings the chain to the beginning of DED2. For CARDS, although their topology is identical with the conserved six-helical bundle fold of the DD superfamily, the structures are unique in that helix H1 tends to be either bent or broken into two closely separated H1a and H1b helices (Figure 2e). In addition, the orientations and lengths of several helices may be somewhat different among the different CARDS. The PYD structures are divergent among themselves in the H2 and H3 region (Hiller et al., 2003; Liepinsh et al., 2003; Natarajan et al., 2006). Although the PYD of NLRP1 has a completely disordered H3, both ASC and ASC2 PYDs have a formed H3 but a long loop between H2 and H3.

There seems to be limited structural plasticity of these domains. Superposition of the Apaf-1 CARD structures in isolation, in complex with caspase-9 CARD and in the context of its NOD domain shows lack of substantial conformational changes (Qin et al., 1999; Riedl et al., 2005). Similarly, superposition of RAIDD DD in isolation and in complex with PIDD DD showed that the structures are similar (Park et al., 2007b; Park and Wu, 2006).

Because of the low sequence homology among DDs, the surface features of these DDs are also entirely different, which may be responsible for their specificity in protein–protein interactions (Park et al., 2007a). However, CARDS seem to be different in this regard. They are mostly polarized with both basic and acidic surfaces, which may be used for protein–protein interactions (Figure 2e). Similarly, PYDs seem to share the surface charge polarization of CARDS.

The few available DED structures seem to have most conserved surface features, which distinguish them from other members of the DD superfamily. The first feature is a conserved hydrogen-bonded charge triad revealed by the high-resolution structure of MC159 (Yang et al., 2005).
Figure 2  Ribbon diagrams for each subfamily of the DD superfamily: (a) Fas DD, (b) FADD DED, (c) Apaf-1 CARD and (d) NLRP1 PYD. (e) Electrostatic surface representation of Apaf-1 CARD. (f) Stick model for the hydrogen-bonding interactions in the charge triad of MC159 DED1. (g) Electrostatic surface representation of MC159 DED1 and DED2, showing the dumbbell-shaped structure, the rich charges on one face and the location of the hydrophobic patch. Panels (f) and (g) are taken from Yang et al. (2005).
The charge triad is formed by the E/D-RxDL (E is Glu, D is Asp, R is Arg, L is Leu and x is any residue) motif and involves the Arg and Asp residues in the RxDL motif in helix H6 and the preceding loop, and an acidic residue in helix H2. Extensive hydrogen-bonding interactions are observed among the charged side chains with the Arg residue situated in between the two acidic residues (Figure 2f). The second surface feature is the conserved hydrophobic patch formed mostly by residues on H2 (Figure 2g). This was first observed in the nuclear magnetic resonance (NMR) structure of FADD DED (Eberstadt et al., 1998) and later shown to be conserved in most tandem DEDs as well (Yang et al., 2005). Both surface features appear to be used for protein:protein interactions.

Interactions in the DD Superfamily

**DD:DD interaction in the Pelle:Tube complex**

The crystal structure of the monomeric Pelle DD:Tube DD complex is the first structure of a complex in the DD superfamily (Xiao et al., 1999; Figure 3a). The biggest surprise from the structure is perhaps the asymmetry of the interaction, considering that symmetric interactions are often expected for homotypic interactions. The first interface between Pelle and Tube involves the H4 helix and the following loop of Pelle and the H1–H2 corner, H6 and the preceding loop in Tube. Most strikingly, the C-terminal tail of Tube wraps around a groove formed by the H4–H5 and H2–H3 hairpins of Pelle to form the second interface and contributes significantly to the interaction (Xiao et al., 1999). Though many charged residues at the first interface are involved in the interaction, three large hydrophobic residues on the C-terminal tail of Tube dominate the second interface.

**CARD:CARD interaction in the Apaf1:procaspase-9 complex**

The only structure of a CARD:CARDCARD complex is provided by the crystal structure of the complex between Apaf-1 CARD and procaspase-9 CARD (Qin et al., 1999; Figure 3b). The interaction is mediated by the mutual recognition of the slightly concave surface of procaspase-9 CARD formed by the positively charged H1a, H1b and H4 helices and the convex surface of Apaf-1 CARD formed by the negatively charged H2 and H3 helices. Three positively charged residues in procaspase-9 CARD and two negatively charged in Apaf-1 CARD are crucial for this interaction (Qin et al., 1999). This study confirmed the ionic nature of the Apaf-1 CARD:procaspase-9 CARD interaction.

**DED:DED interaction in the tandem DED structure of MC159 v-FLIP**

The structure of MC159 revealed the first glimpse of a DED:DED interaction (Li et al., 2006; Yang et al., 2005; Figure 3c). Instead of beads on a string, DED1 and DED2 interact with each other intimately to form a rigid, dumbbell-shaped structure. The two DEDs are related approximately by a translation across the contact interface so that one side of DED1 is contacting the equivalently opposite side of DED2. The translational relationship between DED1 and DED2 is made possible by helix H7 of DED1. The interaction at the DED1:DED2 interface is mostly hydrophobic, mediated by helices H2 and H5 of DED1 and helices H1 and H4 of DED2. There are a total of 195 interfacial atomic contacts, among which 117 are between nonpolar atoms. The interfacial residues, especially those that are completely buried at the DED1:DED2 interface and contribute large surface areas are mostly conserved in tandem DEDs. This suggests that all known tandem DEDs form a similar rigid compact structure as MC159. This interaction between DED1 and DED2 shows some orientational similarity in the Apaf-1 CARD:procaspase-9 CARD interaction.

**Asymmetric oligomeric DD:DD interaction in the PIDD:RAIDD complex**

The PIDD DD and RAIDD DD complex forms the core of the caspase-2-activating complex PIDDosome. Its structure represents the first glimpse of an oligomeric complex in the DD superfamily (Park et al., 2007b). Although RAIDD DD and PIDD DD are monomers, they assemble into a complex that is consistent with a total of 12 DDs. The structure of the complex revealed an entirely asymmetric arrangement that comprises seven RAIDD DDs and five PIDD DDs (Figure 3d). Despite the asymmetry, all DDs in the complex are in quasi-equivalent environments. The structure provided eight unique asymmetric interfaces, which can be classified into three types (Figure 3e). On each DD, three types of interactions together cover a majority of its surface.

In the type I interaction, residues at H1 and H4 of the first DD (type Ia surface) interact with residues at H2 and H3 of the second DD (type Ib surface). This type of interaction is similar to the Apaf-1 CARD:procaspase-9 CARD interaction, although slight orientational adjustment is observed. In the type II interaction, residues at the H4 helix and the H4–H5 loop of the first DD (type Iia surface) and residues at the H5–H6 loop and H6 helix of the second DD (type Iib surface) mediate this interaction. This type of interaction resembles the Pelle DD:Tube DD complex. In comparison with the type I interaction, the type II buries a smaller surface area. In the Pelle DD:Tube DD complex, this interaction is strengthened by an additional interaction between a long tail of Tube and the H2–H3 and H4–H5 region of Pelle. In the type III interaction, residues at H3 of the first DD (type IIIa) interact with residues near the H1–H2 and the H3–H4 loops of the second DD (type IIIb).
Figure 3  Structural features of interactions in the DD superfamily. (a) Pelle DD:Tube DD complex, as a type II interaction. (b) Apaf-1 CARD:procaspase-9 CARD complex, as a type I interaction. (c) MC159 DED1:DED2 interaction, as a type I interaction. (d) Overview of the PIDD DD:RAIDD DD complex in two orthogonal orientations. (e) Superposition of the eight unique interactions in the PIDD DD:RAIDD DD complex. R:RAIDD DD; P:PIDD DD. There are three type I interactions, two type II interactions and three type III interactions. (f) Tetrameric Fas DD:FADD DD complex. Panels (d) and (e) are taken from Park et al. (2007b).
striking, tetrameric arrangement of four FADD DDs bound to four Fas DDs (Scott et al., 2009; Figure 3f). A conformational opening of the Fas DD exposes the FADD DD binding site and simultaneously generates an Fas:Fas interface. Surprisingly, most natural mutations of Fas that cause the human disease ALPS did not map to its interface with FADD DD, raising the possibility that the observed interfaces at pH 4.0 do not represent the physiological interactions.

Preferred Modes among the Homotypic Interactions

There may be preferred modes of interactions among the DD superfamily fold. First of all, almost all structures of DD superfamily complexes are asymmetric, suggesting this to be a preferred means that DDs, DEDs, CARDs and PYDs interact with each other. Secondly, all the observed asymmetric pairs of interactions may be classified into one of the three types of interactions in the PIDD DD:RAIDD DD complex. The Apaf-1 CARD:procaspase-9 CARD interaction and the DED1:DED2 interaction in MC159 may be considered type I whereas the Pelle DD:Tube DD interaction is similar to the type II interaction. In addition, prediction by surface electrostatics has implicated a mode of PYD interaction similar to the observed type I interaction (Liepinsh et al., 2003). Therefore, the observed three types of asymmetric interactions may represent preferred modes of interactions for the entire DD superfamily.

Summary

The DD superfamily is one of the largest and most widely distributed domain superfamilies. One important function of these domains is to participate in homotypic protein:protein interactions in the assembly of oligomeric signalling complexes in apoptosis. Evolutionarily, it seems that the ever-expanding DD superfamily may have evolved by homotypic interactions with other members of each of the subfamilies, these proteins often form the platform of these oligomeric assemblies to allow 'proximity' induced caspase and kinase activation.

Many biochemical and structural studies have been performed on these domains. These studies have revealed a conserved six-helical bundle fold of the DD superfamily. Almost all known protein:protein interactions in the superfamily are either self-association or homotypic interactions with other members of the same subfamily. This is somewhat surprising given the structural similarity among the different subfamilies, but may reflect evolutionary circumstances.

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References


**Further Reading**


