Cell Death and Differentiation (2003) 10, 10–12

www.nature.com/cdd

News and Commentary

Death fold domain interaction in apoptosis

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Cell Death and Differentiation (2003) **10,** 10–12. doi:10.1038/sj.cdd.4401203

The death fold is a structurally defined motif characterized by six to seven tightly coiled α-helices in a 'Greek key fold'[1] (see Figure 1). It is formed by several defined protein-interaction domains, all of which are found on proteins with known or suspected roles in apoptosis and other signaling pathways. Death folds have the interesting feature that they bind to another death fold-containing domain via the same type of protein-interaction domain (homotypic interaction). The four known death-fold domains are the caspase recruitment domain (CARD), the death domain (DD), the death effector domain (DED), and, as recently predicted, the PYRIN domain.[2–6]

The homotypic interactions that characterize the death folds appear to be highly specific, with only two (or in some cases, a few) partners capable of interacting. In every known case, the binding partners have homologous domain (DD–DD, DED–DED, CARD–CARD) and there are no established interactions across groups. The most primitive role for homotypic interactions is obviously that of self-assembly – proteins with such domains have the potential to assemble into larger multisubunit structures composed only of that protein. However, there are no examples of a death-fold interaction in which the domain binds to itself (i.e., to an identical sequence or to another molecule). This suggests that early in evolution the original death fold diverged to ensure homotypic interactions only between slightly different death-fold domains from different molecules. These molecules then came to have very different functions that may nevertheless be related.[7–11]

One danger in the interpretation of structural domains is what we can call the 'functional homology trap.' Although significant homology can suggest a conservation of function, and this can be useful in designing experiments, we simply cannot assume these relations. While several of the death-fold proteins have established roles in apoptosis, immune defense, and/or NF-κB signaling, many of these proteins have unknown or questionable functions. It is probably a mistake to assume that they necessarily have similar physiological roles.

CARD-containing proteins are found throughout the animal kingdom and may be present in plants, fungi, and prokaryotes.[11] In proteins with known roles in apoptosis, CARD domains are present on several mammalian procaspases (caspase-1, -2, -4, -5, -9, -12, -13), the CED-3 caspase in *Cernorabdis elegans*, the Dronc caspase in *Drosophila melanogaster*, and on adapter molecules involved in caspase activation (RAIDD-caspase-2, Apaf-1-procaspase-9, CED-4-CED-3, ARK-Dronc). CARD-containing proteins that may inhibit or perhaps activate caspases (in particular, caspase-1) include ICEBERG,[12] Pseudo-ICE,[13] Cop,[14] and Cardiak.[15] In mammals, CARD domain-containing proteins have a wide range of functions (apoptosis, cytokine processing, immune defense, NF-κB activation), while in insects and nematodes, the CARDs appear to be (so far) restricted to proteins involved in apoptosis.

Probably the best-characterized CARD–CARD interaction is that between Apaf-1 and caspase-9 that activates caspase-9. This interaction is mediated by a CARD present on both Apaf-1 and procaspase-9. A wealth of structural information is in fact available through the determination of solution[16–18] and crystal[1] structure of the isolated Apaf-1 CARD and the crystal structure of the complex with the procaspase-9 CARD.[19] Together with complementary mutagenesis data, this provides a detailed view of the molecular basis governing the regulation of the central machinery of apoptosis, with implications for possible therapeutical interventions.

The CARD domain of Apaf-1 is formed by a bundle of six (or seven) tightly packed α-helices.[1,16–19], closely resembling the overall structure of the RAIDD CARD that interacts with caspases 2 and 9.[20] In the RAIDD CARD only six α-helices are present with Apaf-1 helices H1a and H1b combined into a single helix H1. In the Apaf-1/procaspase-9 complex helices H2 and H3 of Apaf-1 CARD form a convex acidic surface that recognizes a complementary basic concave surface of caspase-9 CARD (formed by H1a, H1b, H4),[19] with residues Y24, D27, S31, D32, Q40, N73,[16,19] (Apaf-1) and R11, R13, R52, R56[19] (procaspase-9) providing specificity in the interaction. Besides these electrostatic interactions, additional hydrophobic contacts are present in the center of the interface thus explaining why the association is refractory to high ionic strength.[16,19,21]

DDs are a death fold found predominantly in the vertebrates, although represented throughout the animals. Several cytokine receptors in the TNF receptor family contain DDs, including the death receptors (TNFR1, CD95, TRAMP/DR3, and the TRAIL receptors). Structurally related DDs are also found on other TNFR family members such as p75 CNTR. Adapter molecules bearing DDs bind to these receptors via specific homotypic interactions (TRADD binds TNFR1, FADD binds CD95, DR3, and the TRAIL receptors). TRADD and FADD also bind each other via DD–DD interaction, but only when TRADD is bound to TNFR1. DD proteins function in both apoptosis and NF-κB signaling in mammals, but apparently only the latter in *Drosophila*.

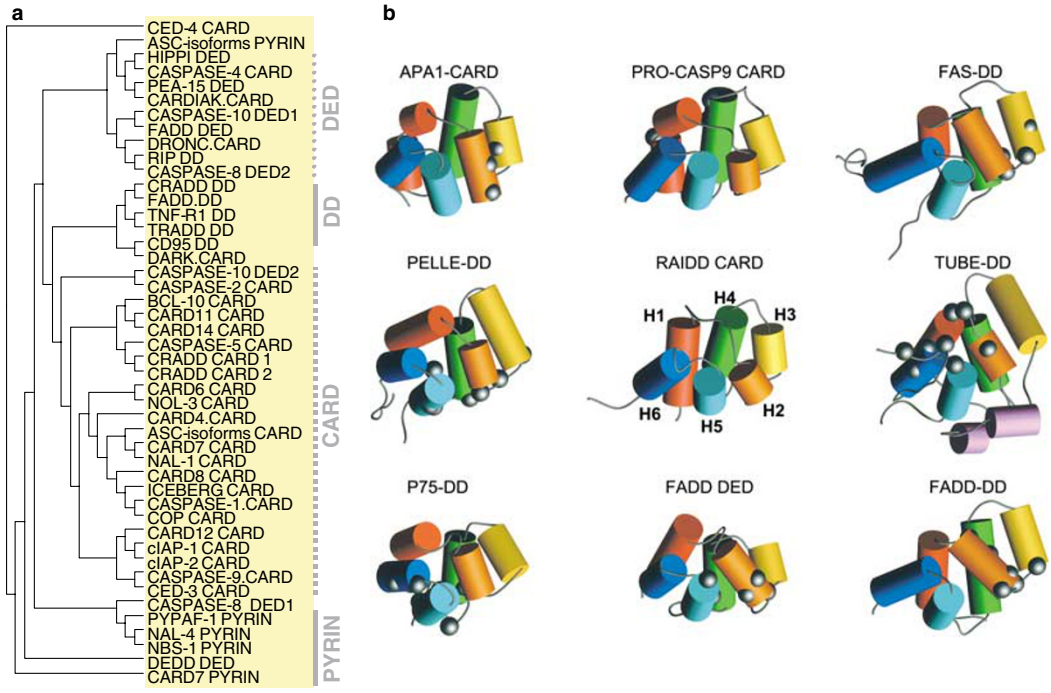

Figure 1 (**a**) Sequence alignment classifies death-fold domains into distinct groups. While some groups show good conservation of function (e.g., CARD domains) other groups show instead considerable functional heterogeneity. (**b**) Superposition of representative 'death fold'[1] domains: CARD, DD, and DED structures as present in the FSSP database.[23] To allow a better comparison the superimposed structures have been separated maintaining their relative orientation. While preserving the central motif of six or seven α-helices, variations in the local structure of the individual domains reflect their adaption to a specific biological function. As a reference individual helices are labeled for the RAIDD CARD (H1, H3, and H4 in the front, H2, H5, and H6 in the back). Structures shown are Apaf-1 and procaspase-9 CARD (PDB[26] entry 3YGS[19]), Fas DD (PDB entry 1DDF),[27] pelle and tube DD (PDB entry 1D2Z),[24] RAIDD CARD (PDB entry 3CRD),[20] p75 DD (PDB entry 1NGR),[28] FADD DED, and DD (PDB entries 1A1Z,[29] 1FAD[30]). Residues important for recognition of the substrate/interaction partner (identified either through structural studies or mutagenesis) are highlighted by spheres. As is evident, the location of the interaction surface area varies and cannot be predicted through structural analogy alone. A wider more general biological function of 'death fold' domains as versatile interaction and adapter modules is indicated by their presence in tube and pelle, two *Drosophila* proteins involved in embryonic development

DEDs, another death fold, are also present on caspases and adapters. FADD bears a DED that interacts with a DED present on caspases-8 and -10, and on the regulatory molecule (it may induce or inhibit caspase activation), c-FLIP. Caspases-8 and -10 each bear two DEDs, and it is possible that these bind to each other intramolecularly to prevent spontaneous association of the caspases with adapters (however this is a speculation). Two other recently identified adapters that activate caspase-8 are HIPPO and HIPPI, which bear DEDs and appear to be involved in caspase activation in response to aggregates of polyglutamine tract proteins such as Huntingtin.[22] The DED-containing caspases function in death receptor-induced apoptosis in mammals (caspases-8, -10), but appear to be involved in NF-κB signaling and antibacterial responses in insects (DREDD).

The PYRIN domain proteins[2,3] are the least understood in terms of possible roles in apoptosis, and no PYRIN–PYRIN interactions have been clearly elucidated or their functions established in death signaling. Nevertheless, and curiously, there is an example of a PYRIN domain protein with a recognizable action: one of the zebrafish caspases carries a PYRIN domain. Therefore, it appears likely that the PYRIN, DED, and CARD domains on caspases all have similar roles in caspase activation.

CARD, DD, DED, and PYRIN 'death fold' domains mediating protein–protein interactions are crucial for apoptosis. Despite their clear functional distinction, comparison of CARD, DD, DED (and the predicted) PYRIN structures (FSSP database)[23] shows a common structural scaffold (Figure 1), also termed the 'death fold'[1] that nature apparently has exploited in distinct ways to perform the associated biological function. Since both the type and spatial location of residues mediating the interactions vary considerably, prediction of functionally important residues based on sequence conservation and structural information will generally not be straightforward and require confirmation by experimental evidence. This is even more the case in considering 'death fold' domains that occur in proteins outside the apoptopic signaling pathways, for example, *Drosophila* pelle and tube[24] (see figure). While being central to apoptosis, the CARD, PYRIN, DD, and DED apparently represent instances of another more general structural motif that controls and mediates an important variety of protein–protein interactions.

Since we do not yet know all of the functions of death-fold proteins, care must be taken in interpreting a confusing and contradictory literature. Proposed functions and binding partners for some of these proteins are based on transient overexpression experiments that may be prone to artifact, and

not all possible functions are ever considered.[8,10,25] Nevertheless, there is clearly a conservation of this structure for the purposes of specific homotypic interactions between different molecules, and as we learn more about the various functions of the death-fold proteins we will gain insights into how these interactions evolved and diverged.

Acknowledgments

We thank Telethon (E872), AIRC (420), the US National Institute of Health, and EU (QLG1-1999-00739; QLK3-CT-2002-01956) for support.

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