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Review / Revue

Fire and death: the pyrin domain joins the death-domain superfamily

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Abstract

Apoptosis and inflammation are important cellular processes that are highly regulated through specific protein–protein interactions (PPI). Proteins involved in these signaling cascades often carry PPI domains that belong to the death-domain superfamily. This includes the structurally well-characterized Death Domain (DD), the Death Effector Domain (DED) and the Caspase Recruitment Domain (CARD) subfamilies. Recently, a fourth member of the DD superfamily was identified, the Pyrin Domain (PYD). Based on sequence alignments, homology to other domains occurring in death-signalling pathways, and secondary-structure prediction, the PYD was predicted to have an overall fold similar to other DD superfamily members. Just recently, NMR structures of two PYDs have been determined. The PYD structures not only revealed the DD superfamily fold as previously predicted, but also distinct features that are characteristic exclusively for this subfamily. This review summarizes recent findings and developments regarding structural aspects of the DD superfamily, with a special emphasis on the PPIs of the DD superfamily. **To cite this article:** *A. Kohl, M.G. Grütter, C. R. Biologies 327 (2004).*

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1. Introduction

Apoptosis and inflammation are two fundamental processes in multicellular organisms that rely on a common set of domains mediating specific PPIs to regulate their signalling pathways [1–3]. Generally, apoptosis is defined as the controlled death of a cell

initiated by either an external or an internal stimulus and showing a specific well-defined morphology of the dying cell. For a living cell, apoptosis is a necessary mechanism during development, when the cell is severely damaged or when it is infected by a pathogen [4]. In Fig. 1a, one of several examples for the extrinsic pathway leading to apoptosis is schematically depicted. In this pathway, apoptosis is initiated by the oligomerised FasL protein binding to its receptor FasR, forcing it to oligomerise. Via its intra-

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cellular Death Domain (DD), FasR recruits the DD of the Fas-associated Death-Domain-containing protein (FADD) in a homotypic PPI. The adapter protein FADD enables caspase-8 to bind to this complex, completing the death-inducing signalling complex (DISC) [5]. This interaction is mediated by a homotypic death-effector domain (DED) interaction between the DED of FADD and the DEDs of caspase-8. In the DISC, the initiator caspase-8 is activated. The activation of initiator caspases requires dimerisation and subsequently proteolytic cleavage [6,7]. Activated caspase-8 is released from the DISC and cleaves the executioner caspases-3 and -7, which themselves cleave many cellular substrates, ultimately leading to the programmed death of the cell [8,9].

The intrinsic apoptotic pathway, shown in Fig. 1b, is also called the apoptosome pathway and engages mitochondria to mediate and integrate the transmitted signals. Bcl-2 family proteins like the BH3-only

proteins BAD, BID, BIM, Noxa and Puma act as initiators in this pathway and on the mitochondria. The multidomain proteins, like BCL-2, BCL-X₁, A1, BCL-W and MCL-1, function as anti-apoptotic regulators. Other multidomain proteins like BAX and BAK are essential components for the execution of the signal. They induce the release of cytochrome *c* and other apoptogenic factors, like SMAC, OMI or AIF from the mitochondria [10]. Upon activation, BAX and BAK oligomerise and lead to a high permeability of the mitochondrial outer membrane by a yet unknown mechanism. This enables cytochrome *c* to diffuse into the cytosol [9], where it is recruited to the apoptotic protease-activating factor 1 (APAF-1). In its monomeric and inactive form, the two WD40 domains shield the caspase recruitment domain (CARD) of APAF-1. Upon binding of cytochrome *c* and dATP/ATP, APAF-1 undergoes a conformational change. The activated form of APAF-1 oligomerises

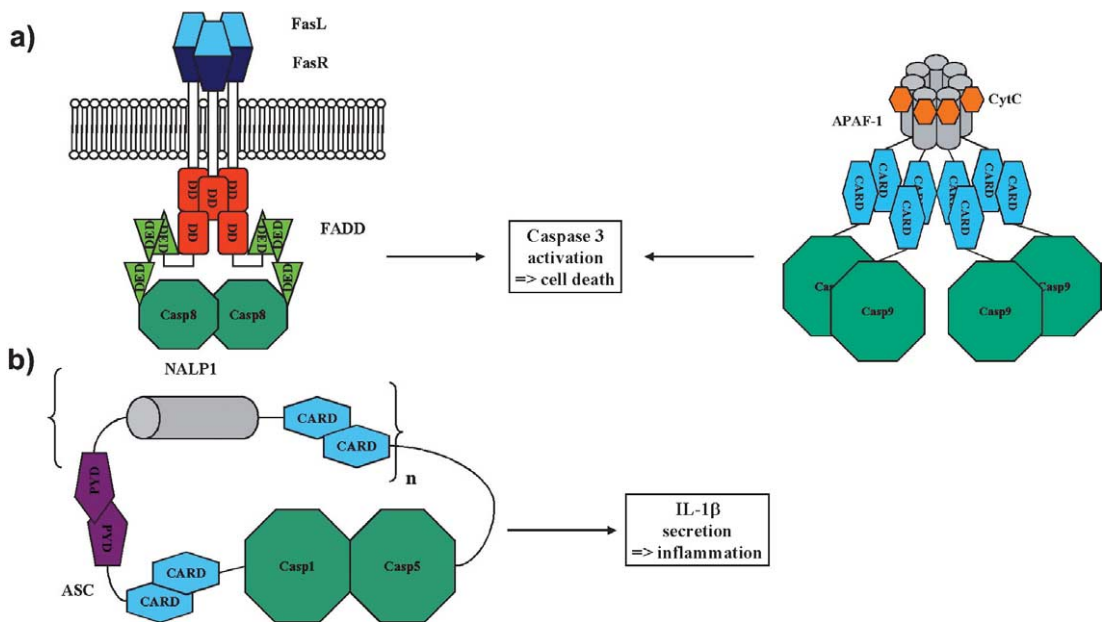


Fig. 1. Protein–protein complexes, in which DD superfamily members play a key role. (a) Left: Schematic representation of the external pathway leading to apoptosis; the death inducing signalling complex (DISC) is shown. At the DISC, caspase-8/-10 is activated, which leads to caspase-3/-7 activation and ultimately to cell death. The oligomeric state of this complex is not known, but it is at least a trimer. The DDs are shown in red, DEDs in light green. Right: Schematic drawing of the heptameric complex of the Apoptosome. Upon binding of cytochrome *c*, the CARD of APAF-1 is released from the WD40 domain and can bind to the CARD of caspase-9. This leads to the activation of caspase-9, which then activates caspase-3/-7 and results in cell death. The CARDS are shown in light blue. (b) Schematic drawing of the inflammasome. The current working model for the assembly and activation of the inflammasome starts with the binding of a stimulus to NALP-1, which results in a domain rearrangement. This enables ASC to bind, hence caspase-1/-5 can bind to the complex and are activated. The active caspases are then able to process pro-IL-1 β to active IL-1 β , which is exported from the cell and acts as an inflammatory signal.

to form a heptameric or higher-order complex with an approximate size of more than 1.0 MDa [9], using its NB-ARC domain (nucleotide-binding domain adapter shared by APAF-1, certain *R* gene products and CED-4) [11]. The unshielded CARD of APAF-1 recruits caspase-9 via its CARD, forming a homotypic interaction. Caspase-9 oligomerises on the apoptosome platform and is subsequently activated. Active caspase-9 then leads to the cleavage of the downstream caspases-3 and -7, resulting in cell death [9]. So far, the apoptosome is the only multiprotein complex involved in apoptosis and inflammation that is structurally characterized. The Cryo EM structure at 27-Å resolution shows a seven-spiked wheel, with the WD40 domains arranged towards the outer end of the spikes and the CARD domain at the inside, forming a knob in the centre of the wheel. Due to the low resolution, a precise positioning of the single domains and a detailed view of the interaction surfaces is not yet possible [12]. For a more detailed description of the apoptosome structure, we would like to refer to a review written by Adams and Cory [13].

Inflammatory processes are initiated and mediated by a closely related set of protein domains as they are used in apoptosis [2]. Similar to the apoptotic pathways, the inflammatory signalling cascades can either originate from an extracellular stimulus that is transmitted to the inside of the cell by a transmembrane spanning receptor, or they are initiated by intracellular sensor proteins. In both cases, the stimulus leads finally to the activation of a caspase or a kinase and ultimately to cytokine release or NF-κB activation. The external pathway uses the Toll-like receptors, which recognize pathogens [14]. The internal recognition of pathogens is mediated by the NALP or NOD proteins, which are multidomain proteins similar in their domain architecture to APAF-1 [15,16]. In general, all these multidomain or Caterpillar (CARD, transcription enhancer, R(purine)-binding, pyrin, lots of leucine repeats) proteins [17] are activated by a common mechanism. First, a small molecule or protein (e.g., LPS, peptidoglycan or a protein, cytochrome c), acting as initial stimulus, is recognized and bound by a detection domain (e.g., LRR or WD40). This induces a rearrangement followed by the sequestering of an ATP/GTP to the NTP binding and oligomerisation domain (NB-ARC [11], NACHT [18]), which then oligomerises to form a stable multimeric plat-

form. In a subsequent step, other proteins, like kinases or proteases are recruited to this platform and are activated. In the case of the NOD1/2, a CARD-containing kinase RIP2 attaches itself directly to the NOD CARD, is activated and leads to the activation of NF-κB [19]. For the NALP superfamily, the story is again more complicated. So far 14 NALPs have been identified in the human genome. They all possess an N-terminal Pyrin Domain (PYD) and, in the case of NALP-1, a C-terminal CARD. For NALP-2–14, an additional protein named Cardinal plays the same role as the C-terminus of NALP-1. Fig. 1b shows a schematic drawing of the current model for the NALP-1 inflammasome, a multiprotein complex with a molecular size well above 700 kDa [20,21]. In the inflammasome, NALP-1 is thought to interact with a not yet identified stimulus through its leucine-rich repeat domain. It is assumed that this induces a structural rearrangement, which unshields the PYD and CARD domains. The NACHT domain of NALP-1 was identified to be a similar nucleotide-binding domain as the NB-ARC domains in APAF-1. Therefore, it is believed that NACHT binds ATP or GTP and mediates the oligomerisation of NALP-1 in analogy to the NB-ARC domain of APAF-1. In this oligomerised form, the adapter protein ASC can interact with NALP-1 using the PYDs of both proteins. With its second domain (a CARD), ASC is able to recruit caspase-1/-5, which is activated through this oligomerisation. A similar process can take place at the C-terminus of NALP-1, where caspase-1/-5 can directly interact with the CARD of NALP-1 [20]. It is assumed that a similar activation mechanism holds true for NALP-2 to 14, even though the additional protein Cardinal might alter the details to some extent [21]. Ultimately, all NALP inflammasomes lead to the activation of a caspase. In the NALP-1 inflammasome, this is predominantly caspase-1, with its main substrate pro-IL-1β. Active caspase-1 cleaves pro-IL-1β to release active IL-1β, a potent marker for inflammatory processes [20]. A closely related system is the PIDDosome, a complex formed by the platform protein PIDD, the adapter RAIDD and caspase-2 [22].

Except for the above-described DISC, apoptosome and inflammasome, there are now many more signalling complexes known, in which domains of the DD superfamily play a crucial role. Proteins carrying a DD, DED, CARD or PYD are known from the TNF

[9], the TRAIL [9] or the TOLL-like receptor extrinsic pathways as well as from the NOD signalosome [19] or the PIDDosome [22], which are intrinsic signalling pathways.

2. General domain architecture

All four DD subfamilies share a common fold and domain architecture. The common motif is an antiparallel six-helix bundle of amphipathic helices, with an approximate size of 85–100 amino acids. The individual length and relative orientation of the six α -helices is different in every subfamily. Even within one subfamily, the helix orientation between the family members can be rather different (see CARD subfamily). The sequence identity among the four subfamilies is between 10% and 25%, well below the threshold of 25 to 30% required for reliable homology modelling (Fig. 2). Even within one subfamily, the sequence identities are usually low and only functional residues or residues that define the hydrophobic core are conserved. This implies that experimental structure determination is a prerequisite for a detailed structure function analysis and PPI studies of these protein modules. The often-applied homology modelling is a not reliable option in this case. The fact that individual domains might differ significantly compared to the general fold is a consequence of the low sequence conservation and leads to the relatively high plasticity of the DDs. A good example of change event in secondary structure elements between different members of one subfamily can be found in the CARDS. Another example of high plasticity within a subfamily is the PYD (discussed in the following sections, CARD and PYD).

So far, mainly homotypic interactions for the DD superfamily are known. Even though the interaction of PEA-15 with its substrate shows that the DDs are capable of interacting directly with other proteins [23]. Generally, two interaction modes are possible: the self-interaction of two identical domains and the interaction of two different domains of the same subtype. So far, there is no evidence on a structural level that two domains of different subfamilies interact with each other.

3. DD subfamily

DDs are mainly found in proteins involved in apoptotic- or apoptosis-related pathways and in NF- κ B signalling. The spectrum of DD-containing proteins includes TNF receptors like Fas, the TNFR, TRAMP and TRAIL receptors, adapter molecules like FADD, TRADD and MyD88, but also proteins like the IRAK kinase family and the cytoskeleton ankyrin proteins. DDs are predominantly found in animals, extending from human to the fruit fly and the nematode *Ceanorhabditis elegans*. To date, no DDs have been identified in proteins from plants, fungi or prokaryotes, except for one example in the bacterium *Oceanobacillus iheyensis*, in which the hypothetical protein Q8EL73 is predicted to contain a C-terminal DD. Currently 227 different proteins with a DD are listed in the InterPro database [24]. Structurally, the DD subfamily is well characterized by five NMR structures (1DDF [25], 1E3Y [26], 1FAD [27], 1ICH [28], 1NGR [29]) and the crystal structure of a complex (1D2Z [30]). In more details, the six-helix bundle of the DD subfamily can be described as an arrangement of two, three-helix bundles (helix 1, 5, 6 form

DD		
mFAAD	89–183>	AAPPGEAYLQVAFDIVCDNVG- RDWKRLARELKVSEAKMDGIEEKY PRS- LSEVRRESLKVWKN AE-KKNASVAGLVKALRTRCRNLNLVADIVEEAQES-----
hFAS	224–318>	LSDVDLSKYITTIAG---VMTLSQVKGfVRKNGVNEAKIDEIKNDNVQDTAEQKV-QLLRNWHQLHGKKEA- YDTLIKDLKKNLCTLAEKIQTIILKDI -----
DED		
hFADD	1–083>	MDPFIVLLHSVSS LSSSETELKYLCLGRVGRKRLERVQSG-- LDLFSMLLE QNDLEPGHT--- ELLRELLASLRHRD LLRRVDDFE-----
CARD		
hICEBERG	2–090>	ADQLRKKRRIFTHSVAG AGTINALDCLLE DEVIS QEDMNVKVR DENDT- VMDKARVLIDLV TGKGP KSCCKFIKHL CEED-PQLASKMGLH-----
hAPAF1	1–095>	MDAKARNCLLQHQREALKDIKT--- SYIMDHMSDG FILT ISEEEKVR NEPTQ-- QQRRAAMLKML KKDND SYVSYFNALL HEGYKDLAALLHDGIPVVS---
hCASPASE9	1–095>	MDEADRRLLRRCRLRIVEELQV---DQLWDVLLSRELFRPHMEDIQRAGSGSRRDQARQLI IDLETRGSQALPLFIS CLEDTGQDMLASFLRTNRQA----
PYD		
hASC	1–089>	--MGRARDAILDALENL- TAEELKFKFK LKLS-VPLREGYGRIPR GALLSMDALD LTDKLVSFY LETYGAELTANVLR DMGL QEMAGQLQ AAT-----
hNALP1	1–090>	--MAGGAWGR LACYLEFL-KKEELKEFQL LANKAHSRSSSGETPAQPEKTS G-MEVASYLVAQYGEQ RAWDLALHTW EQMGLRSLCAQ AQEGA-----

Fig. 2. Structure-based alignments of all four subfamilies of the Death Domain Superfamily. The death domain (DD) is followed by the death effector domain (DED), by the caspase recruitment domain (CARD) and by the pyrin domain (PYD). The sequences were extracted from the corresponding PDB files that have been obtained from the protein database. The structural alignment was generated using the SPDBviewer [50]. Helical regions are shown in bold and italics.

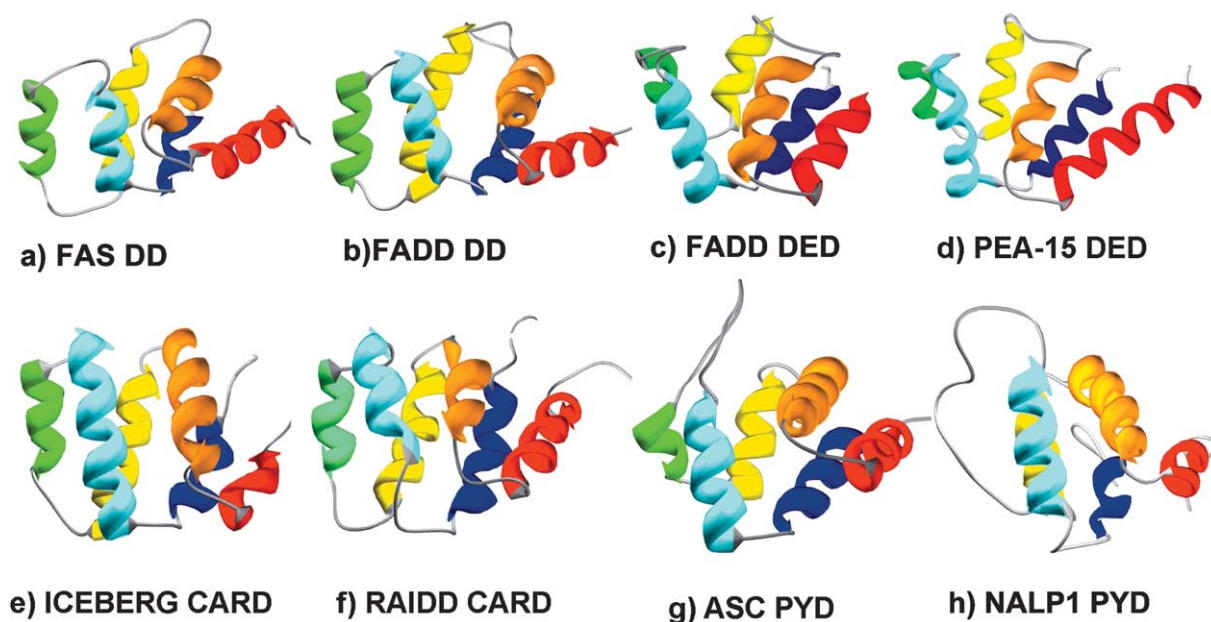


Fig. 3. Ribbon representation of the death domain superfamily. The helices 1 to 6 are shown in blue, cyan, green, yellow, orange, and red, respectively. All proteins are superimposed and in the same orientation. DD of (a) mFADD and (b) hFAS. DED of (c) hFADD, and (d) cgPEA-15. CARD of (e) hICEBERG and (f) hRAIDD. PYD of (g) hASC, and (h) hNALP1.

the first bundle, helix 2, 3, 4 form the second bundle), which are rotated approximately 45 to 90° with respect to each other (Fig. 3a and b).

4. DED subfamily

Proteins containing a DED are found primarily in vertebrates, but also in viruses. The most important DED-containing proteins are certainly the initiator caspases-8 and -10, the adapter molecule FADD and the cellular and viral FLIPs. Despite their importance, only two NMR structures (1A1W [31], 1N3K [23]) (Fig. 3c and d) have verified that the DEDs are indeed members of the DD superfamily. Currently there are only 44 DED-containing proteins listed in the InterPro database. In contrast to the DDs, the architecture of DEDs displays a more parallel arrangement of the six helices with only helix 2 and 3 slightly tilted with respect to the rest of the domain (Fig. 3c and d). DEDs are structurally not as well characterized as DDs, mainly because DEDs seem to have a more hydrophobic surface and are therefore difficult to express, isolate and characterize due to their tendency to aggregate.

5. CARD subfamily

Biophysically, the best-characterized subgroup in the DD superfamily is the CARDs [32]. CARD-containing proteins are found throughout the animal realm, in viral and even prokaryotic proteins. It is therefore expected that they exist also in plants and fungi. CARDs were identified in proteins with different functions and very different domain architectures. The so-called Caterpillar proteins [17] like APAF-1, NOD-1, NOD-2 and NALP-1 contain a CARD as well as the caspases-1, -2, -4, -5, -9 and -12. The group of CARD-containing proteins extends further to kinases like RIP-2, adapter proteins, like ASC, and CARD only proteins, like Iceberg. In total 135 different proteins are annotated to contain a CARD in the InterPro databank. The protein databank currently comprises four NMR structures (1C15 [33], 1CWW [34], 3CRD [35], 1DGN [36]), two crystal structures (1CY5 [37], 2YGS [38]) (Fig. 3e and f) of single domains and one crystal structure of APAF-1 in complex with caspase-9 CARD (3YGS [38]). The CARD architecture is characterized by the most parallel arrangement of the six helices compared to the helix arrangement in all the

other DD subfamilies (Fig. 3e and f). A special feature of the caspase-9 and the APAF-1 CARD is the kink in helix one, which splits it into two smaller helical segments. CARD containing proteins are mediating PPIs in inflammation, innate immune response, cytokine processing, apoptosis, and NF- κ B signalling.

6. PYD subfamily

The most recently discovered member of the DD superfamily is the Pyrin domain (PYD, PAAD, DAPIN). Based on sequence alignments, homology to other proteins and secondary structure predictions, three groups [39–42] suggested that PYDs belong to the DD superfamily. A variety of different PYD-containing proteins with different functions, in different pathways were identified in the last few years. The Caterpillar proteins NALP-1 to 12, the adapter protein ASC, the protein Pyrin, the PYD only protein ASC2/POP1, several viral PYDs and even two zebra fish caspases were all identified to contain a PYD [43]. Presently, the PYD subfamily is the least well characterized of all four subgroups. Proteins carrying a PYD domain are mainly involved in inflammation, apoptosis and NF- κ B signalling. So far, 62 proteins are listed to contain a PYD in the InterPro database, mainly from species belonging to vertebrates and viruses. Only two recently published NMR structures (1PN5 [44], 1UCP [45]) are available to date, which show that PYDs belong to DD superfamily. The ASC and the NALP-1 PYD structure show basically the same overall features, the well-known six-helix bundle (Fig. 3g and h). A clear difference between the DD superfamily and the PYDs is seen in the helix/loop three region. In the ASC structure, helix 3 is still present but is only formed by a small stretch of four amino acids preceded by a long, rather flexible loop (Fig. 3g). The NALP-1 structure is not showing any regular secondary structure in this region. Indeed, helix 3 in the NALP-1 NMR structure is replaced by a flexible loop (Fig. 3h). Whether the loop three rearranges to form a helix under certain conditions or upon binding is not yet known, but is a possibility. The flexible, elongated loop region preceding helix 3 seems to be a common feature in many PYDs except for the viral sequences. The overall arrangement of the helices in the PYDs is similar to those found in the DDs. As in the DDs,

the PYDs can be described as two three-helix bundles having a tilt of 45 to 90° with respect to each other.

7. General mode of interaction

In this section, the current literature and data regarding the different interaction modes within the DD superfamily are reviewed. From the available data, it is assumed that each DD superfamily member interacts exclusively with another member of the same subfamily in a so-called homotypic interaction. Mutagenesis data and model considerations suggest that domain–domain interactions are compatible with either a dimeric or possibly a trimeric arrangement. So far, only crystal structures of dimeric arrangements of DD superfamily members have been determined. Crystal structures providing a detailed picture of the domain interaction modes are only available for the DD [30] and CARD [38] subfamily. Our understanding of DED [46] domain interactions is only based on modelling and mutagenesis of surface residues. At the moment, no structural information is available regarding the PYD-domain interactions and only cell biology data supports that PYD-domain interactions really exist, in, e.g., the inflammasome [20].

8. DD–DD interaction

The crystal structure of PELLE a *Drosophila* kinase and TUBE an adapter protein (1D2Z [30]) illustrates the characteristic DD interaction features on a molecular level, Fig. 4a. PELLE and TUBE are part of an extrinsic signalling pathway starting with the receptor Toll and leading to the phosphorylation of Cactus, an I- κ B homologue. The PELLE DD interacts mainly via two patches: in the first one, residues located in helix 4 and the following loop are involved; in the second one, the cavity between helices 4 to 5 and the hairpin between helices 2 and 3 are utilized. The first patch of PELLE interacts mainly with a surface on TUBE formed by a groove between helices 1 and 2 and, on the other hand, by helix 6 and the preceding loop. The second patch on PELLE interacts mainly with the C-terminal extension of TUBE (Fig. 4a). The buried accessible surface area (Δ ASA) for this complex is about 2000 Å², which is in the normal range for a heterodimeric PPI. The two het-

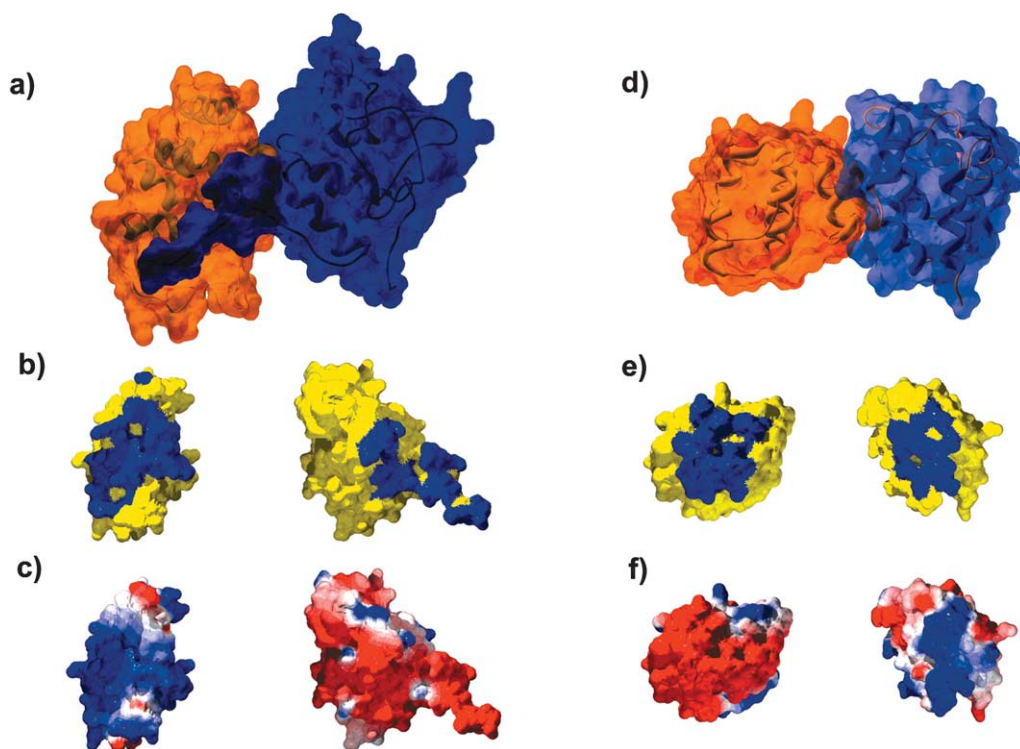


Fig. 4. Solid surface models of the two known DD superfamily complex crystal structures. (a) Complex of PELLE (blue) and TUBE (gold) DD. (b) Representation of the interacting residues. The complex is opened up like a book, the surface is coloured yellow, interaction residues are coloured in blue. (c) The electrostatic potential was calculated and projected onto the surface, blue represents a positive, red a negative potential. The interaction of PELLE and TUBE DD clearly uses an electrostatic component. (d) Complex of APAF-1 (gold) and caspase-9 (blue) CARD. (e) Surface representation of the interaction coloured like in (b). (f) Representation of the electrostatic potential, coloured like in (c). The electrostatic component is very important for the overall interaction in the CARD–CARD complexes.

erodimers in the asymmetric unit of the crystal structure of PELLE and TUBE are not identical. A superposition of both heterodimers shows a perfect fit of the PELLE-DD and a relative rotation of approximately 7° for the TUBE-DD between the two heterodimers. Despite the differences, both heterodimers share the same interaction surface [30]. This variability in the complex formation in combination with the high tolerance for certain point mutations in the interface shows the rather flexible nature of this interaction surface.

The available mutagenesis data, particularly for the FAS/FADD or the TNF/TRADD system, cannot be fully explained assuming a similar structural arrangement, as seen in the PELLE and TUBE complex structure. Therefore, it was suggested by Weber and Vincens that the DDs form a trimeric arrangement

[47] (Fig. 5). The model for this trimeric arrangement was derived by combining the information of the two available crystal structures of APAF-1 with caspase-9 and PELLE with TUBE. A superposition of the available crystal structures of the complexes of APAF-1/caspase-9 (termed type 1 interface) and PELLE/TUBE (type-2 interface) resulted in a trimeric arrangement with a new type-3 interface (Fig. 5). Superimposing and exchanging the APAF-1, caspase-9, PELLE and TUBE molecules with the available NMR structures of Fas-DD and FADD-DD led to the proposed potential trimer of DDs in the DISC [47]. In addition, a higher-order hexameric arrangement of the DDs in the DISC was proposed, based on the trimer [47]. The resulting Fas/FADD trimer fits the available mutagenesis data for this system surprisingly well. New mutagenesis studies by Hill et al. [48] also seem

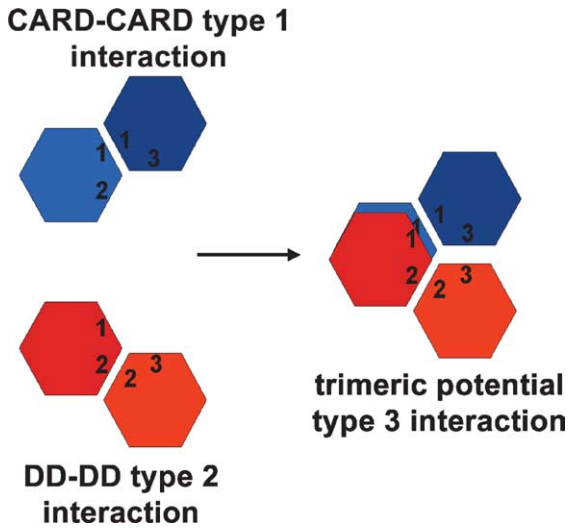


Fig. 5. Schematic drawing to explain the generation of the trimeric model for the Fas-DD and FADD-DD system [47]. Caspase-9 CARD is shown in light blue, APAF-1 CARD in blue, PELLE-DD in red and TUBE-DD in orange. Superimposing caspase-9 CARD to PELLE-DD results in a potential new type-3 interaction surface between APAF-1 CARD and TUBE-DD. The trimeric DED model was derived using the trimeric DD model and replacing the DDs by DED structures, also taking into account the stoichiometry of DEDs in the DISC [46].

to support this model. To ultimately verify these proposed models, a structure of such a trimeric complex is required. Nowadays, it is assumed that the DISC is a dynamic assembly and in continuous change. Therefore, a structure of such a trimeric or higher-order arrangement is a challenging task.

9. DED–DED interaction

Structural data showing DED–DED interactions are not available to date. Based on the NMR solution structures of the FADD-DED, the PEA-15 DED, and on mutagenesis studies, possible DED–DED interaction modes were modelled. Kaufmann et al. [46] proposed a trimeric DED interaction model in analogy to the higher order DD interaction model [47]. This DED model was experimentally verified by *in vivo* mutagenesis studies. It was shown that a basic stretch of amino acids on helix 3 of FADD-DED is needed to form the DISC. According to this model, helix 3 of FADD-DED would be engaged in a type-3 interface. Together with the PEA-15 DED [48] structure, a

mutagenesis study was published, which showed that residues in the helices 1, 2, 5, 6 and in the C-terminal tail are needed for binding to the interaction partner. It was also shown that the PEA-15 DED interacts with its interaction partner as does TUBE with PELLE. Unfortunately, the PEA-15 interaction partner is not a DD superfamily member that somehow limits the implication for the DD or DED homotypic interactions. It also shows that DD superfamily members are not limited in their interactions with the DD superfamily members.

10. CARD–CARD interaction

Interactions in the Apoptosome are CARD–CARD contacts between APAF-1 and caspase-9. This interaction was intensively studied by site directed mutagenesis and is illustrated best by the crystal structure of the complex (3YGS) [38] (Fig. 4b). The APAF-1 CARD donor interacts with the caspase-9 acceptor CARD via residues that are located mainly in helices 2 and 3. The residues form a patch on this slightly convex acidic surface, which interacts with another basic patch on the concave surface on caspase-9 CARD (Fig. 4b). The important interaction residues in the caspase-9 CARD are located in the helices 1 and 4. In addition to the electrostatic interaction, there are also hydrophobic contacts present in the CARD–CARD complex. The buried accessible surface area (Δ ASA) in the APAF-1/caspase-9 complex covers about 1100 Å², which is much smaller than in the PELLE–TUBE complex, but still within standard values for heterodimeric PPI. Mutagenesis studies do not support a model with a second interface, like in the case of the DDs or the DEDs.

11. PYD–PYD interaction

Currently, the only evidence for PYD-domain interactions is based on cell biology data in, e.g., the inflammasome [20]. Prior to the publication of the two PYD NMR structures, Gozik et al. modelled the PYD–PYD interaction, using homology models of PYDs [49]. In this model, interaction and binding are mediated primarily by complementary surface charges, in analogy to the APAF-1/caspase-9 CARD complex structure. The now-available two PYD structures revealed a modified DD fold and a different surface,

in contrast to what was predicted simply by homology modelling. Important differences are seen in the loop/helix 3 region in the PYD in comparison to the DD superfamily. Loop/helix 3 in the PYDs is either only a short α -helix with a preceding long loop [44] or not structured at all [45]. Models that predicted PYD-domain interactions based either on the DD-complex or the CARD-complex must take into account that loop/helix 3 would play a critical role in any interaction. Therefore, data from mutant PYDs affecting the PYD–PYD binding or a structure of a PYD–PYD complex are needed to provide a more precise view of this important interaction.

12. Conclusions

Despite the availability of biochemical and structural data regarding the DD superfamily, major questions remain still unanswered. We are still missing structures showing the homotypic interactions in the DED and PYD subfamilies. Current models are a first step, but do not provide a precise-enough picture. An even more challenging task is to unravel the higher-order arrangements of the DD superfamily members in multiprotein complexes like the apoptosome, DISC, and inflammasomes.

Note added in proof

Nam et al. have recently published an article, showing that heterotypic DD fold interactions are possible, Y.J. Nam, K. Mani, A.W. Ashton, C.F. Peng, B. Krishnamurthy, Y. Hayakawa, P. Lee, S.J. Korsmeyer, R.N. Kitsis, Inhibition of both the extrinsic and intrinsic death pathways through nonhomotypic death-fold interactions, *Mol. Cell* 15 (2004) 901–912. In this paper the authors demonstrate that a DD interacts directly with a CARD, and it therefore offers a new perspective, contradictory to the classical view of only homotypic DD fold interactions.

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