

The death domain: a module shared by proteins with diverse cellular functions

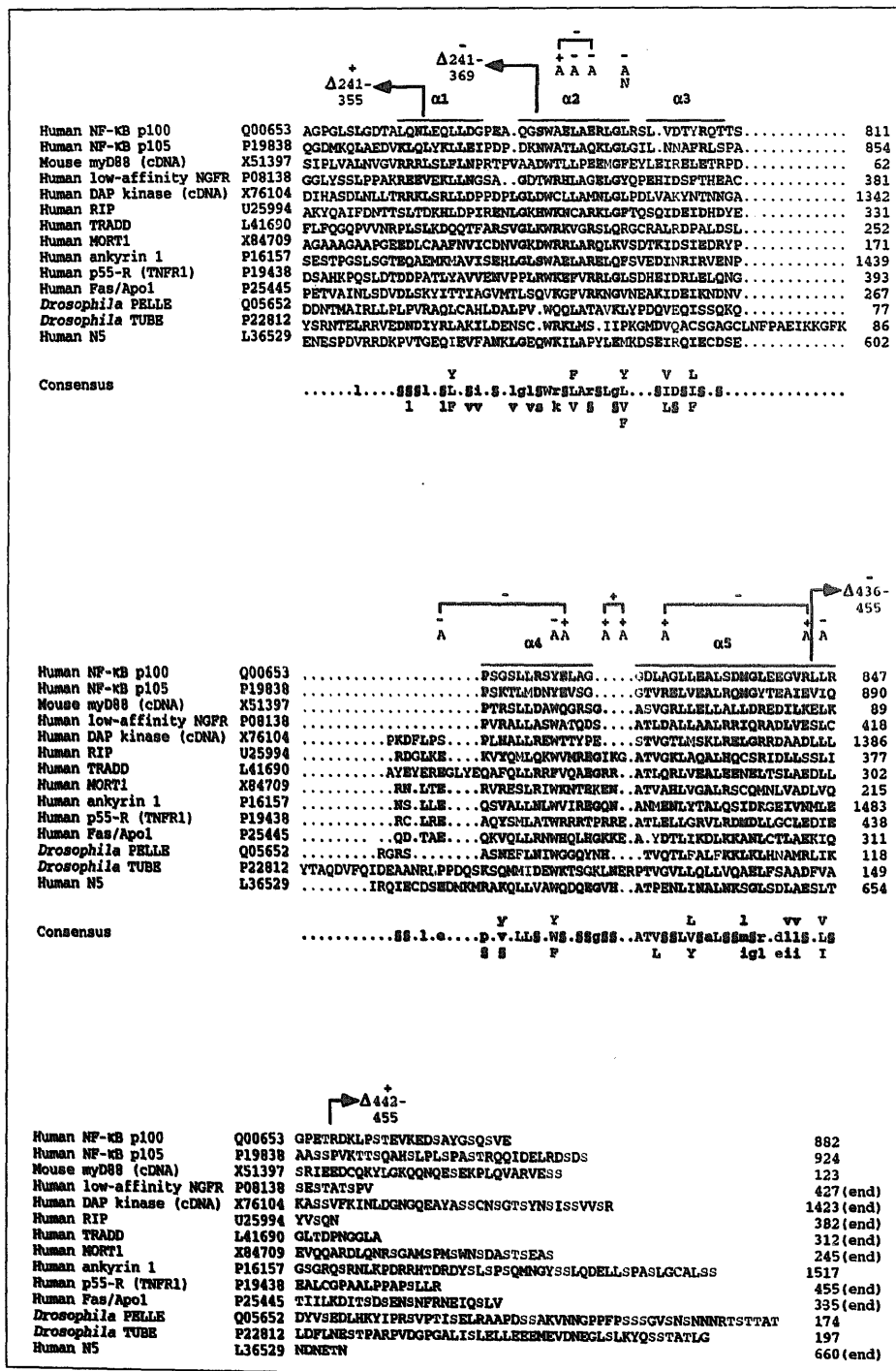
The death domain was initially described as a region of similarity within the intracellular portions of the 55 kDa tumour necrosis factor (TNF) receptor (p55-R) and Fas/Apo1 (whose ligand, FasL, is a TNF-family member), essential for the transduction of cytotoxic signals¹⁻³. Later,

these regions were found to be involved in protein-protein interactions⁴ based on homo- and heterodimerization. This property has enabled several novel death-domain-containing proteins to be identified by means of the yeast two-hybrid system [MORT1 (Ref. 5), also named FADD⁶; RIP⁷; and TRADD⁸]. Other proteins containing death domains - the low-affinity nerve growth factor receptor (NGFR)⁵ and the ankyrins^{5,9} - were discovered using sequence alignment techniques. Here, we show that death domain sequences also occur in several additional proteins and that there are

probably two subtypes of this motif. Subtype 1 occurs in Fas, p55-R, MORT1/FADD, ankyrin 1, RIP and TRADD, as was previously summarized in Ref. 9. To this list we add two *Drosophila* proteins, the serine/threonine kinase PELLE¹⁰ and its potential activator TUBE¹¹, and N5, a human nuclear matrix protein that co-localizes to the centres for RNA processing and can bind to the amino terminus of the retinoblastoma protein¹². Subtype 2 was revealed by screening protein databases for sequence similarities to DAP kinase, a novel serine/threonine kinase that functions as

Figure 1

Alignment of various death domains and their flanking regions. Alignments were performed using the LineUp program of the GCG program package. The consensus sequence is shown at the bottom of the alignment. The core conserved amino acids are shown in red and in upper case in the consensus sequence. More variable amino acids are shown in blue and are lower case in the consensus sequence. Green residues show conserved charged or polar residues, which are represented as § in the consensus. Serine and threonine residues located downstream of the death domains are shown in yellow. The positions of the predicted helical structures are indicated over the aligned sequences as α 1-5. Inactivation mutations and deletions introduced in the TNFR1 (P55-R) death domain are also indicated (compiled from Fig. 3 in Ref. 3); amino acids that were converted to alanine are labelled A. The site of the *lpr^{ck}* mutation in the Fas receptor and analogous mutations introduced in the TNFR1 and MORT1 death domains is indicated by N. A minus sign immediately over the letters A and N indicates that TNF-R1 could not signal cytotoxicity when this single amino acid replacement was made. A minus sign immediately over the bracket indicates that TNFR1 could not signal cytotoxicity when the corresponding group of amino acids was mutagenized. The death domain sequences are conserved in all cloned identical proteins from other species as well as in various types of ankyrins, including those from *Caenorhabditis elegans* (unc-44). The death domain of myD88 was found to start in a region that was previously considered to be a 5'-untranslated region, yet it contains an open reading frame that is an extension of the proposed one. This could suggest that the start point of the protein has not yet been defined.



a positive mediator of interferon- γ -induced programmed cell death¹³. A search of the SBASE¹⁴, SWISS-PROT, PIR, GenPept and Prodom¹⁵ databases using the BLAST program^{16,17} (scoring matrix, Blosum62) revealed that the very carboxy-terminal portion of DAP kinase has significant homology to regions within the cytoplasmic domain of the NGFR, the p100 and p105 members of the Rel protein family and a primary myeloid differentiation response gene product called myD88.

The region within the NGFR that displayed homology to DAP kinase was the same region as that found to be homologous to the death domain⁵. Therefore, it was appealing to compare the two groups of proteins with each other. The pairwise relationships among proteins comprising those groups are summarized in Fig. 1. The similarity among the aligned sequences confirmed the presence of the death domain in all 14 proteins and indicated that the region of homology spans about 90 amino acids. Notably, the borders of the death domain that are proposed here, on the basis of sequence similarity, coincide almost exactly with the experimentally determined boundaries of the region within the cytoplasmic domain of the p55-R that is necessary for conferring cytotoxicity³ (Fig. 1).

Analysis of secondary structure using the PHD program^{18,19} indicated that all 14 death domains are composed of five sequential α -helices, predicted with the highest probability and interrupted by loop regions. (Helix 1 was not detected in Fas and the TNF receptor, whereas helix 2 was not observed within the corresponding portion of myD88. N5 lacks a structurally defined helix 4, and Helix 3 was not detected in NGFR, myD88, MORT1 or TUBE.) Notably, the predicted helical regions within various death domains contain the clusters of amino acids that display the most prominent homology. Interestingly, almost all the artificial mutations in the TNF receptor death domain shown to interfere with cytotoxic signalling by this receptor fall

on conserved amino acids within the defined α -helical structures³ (Fig. 1). The same is true for the naturally occurring *lpr*^{CB} mutation that ablates the function of Fas/Apo1 and prevents its binding to MORT1/FADD^{5,6,20}.

The most prominent features that distinguish the death domains of both subgroups are: (1) lesser conservation of helix 1 in subgroup 1 death domains; (2) longer intervening regions between helices 3 and 4, and 4 and 5 in subgroup 1

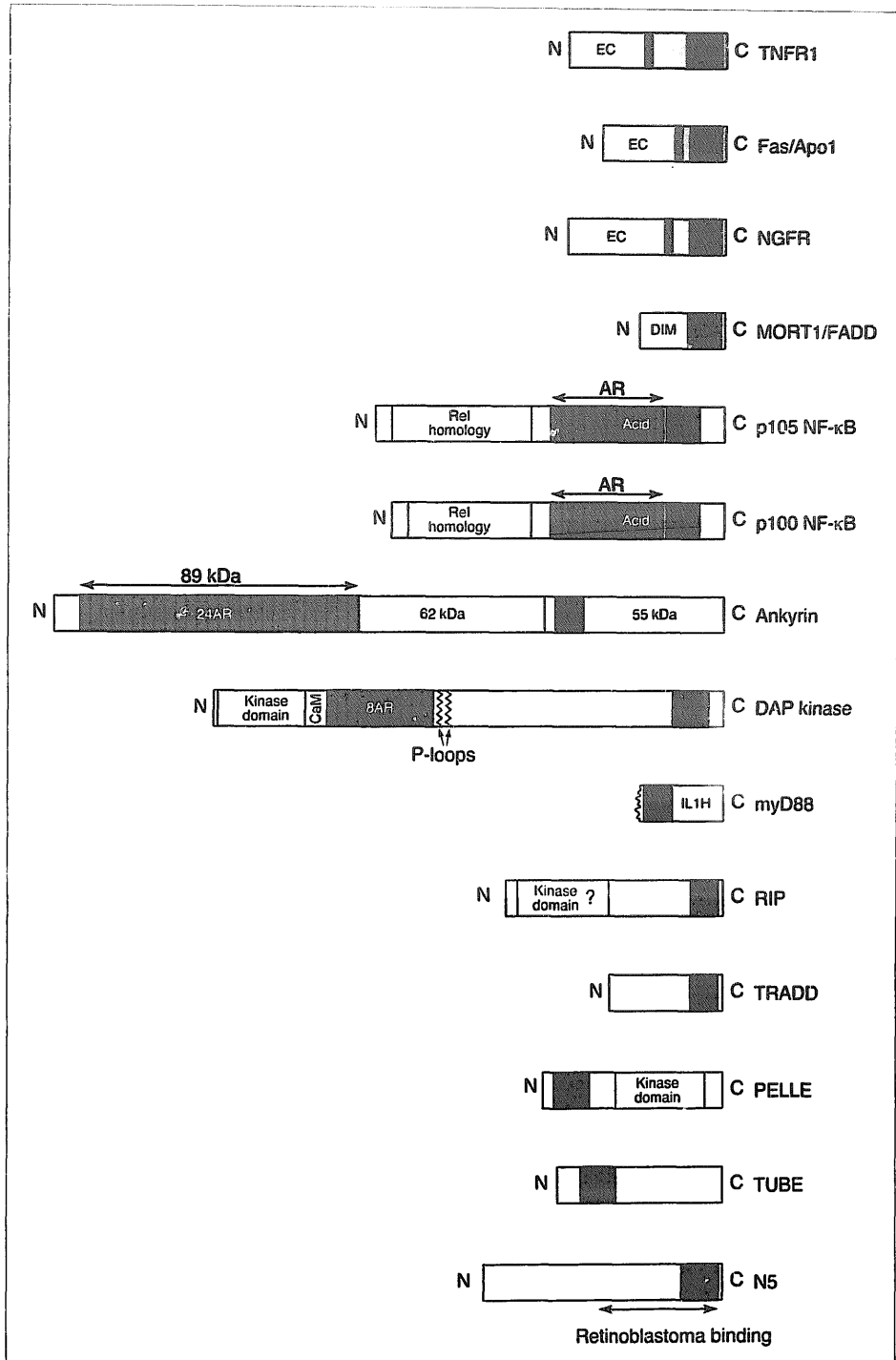


Figure 2

Schematic representation of the death-domain-containing proteins. Death domains are shown as black boxes. Red boxes represent ankyrin repeats (AR). Transmembrane regions are shown in green and intracellular portions of receptors in yellow. Zig-zags represent P-loop motifs. Also shown are: the calmodulin-binding domain (CaM) of the DAP kinase; the IL1/Toll receptor homology domain (IL1H)^{26,27}; the dimerization domain⁵ of MORT1/FADD (DIM); the acidic transactivation domain within p100 and p105 (Acid)²⁸; and, in ankyrin, the anion exchange protein-binding domain (89 kDa), the spectrin-binding domain (62 kDa), and a domain that regulates the binding of ankyrin to spectrin and the band 3 protein (55 kDa)²⁹.

death domains (here DAP kinase and PELLE display features that fit both subgroups); (3) the presence of two pairs of conserved proline residues in the regions between helices 1 and 2, and 3 and 4 in subgroup 2. It is noteworthy that although helix 1 is less conserved within subgroup 1 and was not even predicted to exist within two members of this group (Fas/Apo1 and p55-R), this region was proved to be necessary experimentally for signalling p55-R-mediated cytotoxicity³. When protein databases were searched (using BLAST) with consensus sequences derived from these two subgroups separately, only members of the appropriate subgroup were detected. A single exception relates to the ankyrins, which could be detected by consensus sequences of both subgroups. When consensus sequences of both subgroups were aligned, they revealed three boxes of homology that coincide in position with helices 2, 4 and 5 (not shown).

In ten out of 14 proteins, death domains are localized at their extreme carboxyl terminus (Fig. 2). In addition, almost all death domains (except those of p55-R, RIP, TRADD and N5) are followed by a serine/threonine-rich region (Fig. 1). This implies that these sequences might impose a regulatory effect (upon phosphorylation or dephosphorylation) on the function of the death domain. Indeed, it was demonstrated that the most carboxy-terminal 15 amino acids of Fas/Apo1 negatively regulate its signal transduction².

Seven of the death-domain-containing proteins are directly involved in induction of cell death. p55-R, Fas/Apo1 and NGFR are members of the same superfamily of TNF/NGF growth factor receptors. Fas/Apo1 and p55-R trigger destructive activities in cells upon stimulation by their ligands, whereas the NGFR induces cell death upon ligand withdrawal²¹. MORT1/FADD, RIP and TRADD were shown to kill cells when overexpressed⁵⁻⁸. The gene encoding DAP kinase was cloned as a positive mediator of programmed cell death whose reduced expression conferred resistance to cell killing by interferon- γ ¹³. Although there are no direct indications that p105 and p100 NF- κ B are involved in death signalling, one of the members of the same gene family, *c-rel*, displayed high levels of expression at the sites of programmed cell death in the developing avian embryo²². Moreover, NF- κ B activation occurs after either treatment of responsive cells with TNF- α ²³ or overexpression of p55-R or TRADD death domains⁹. So far there is no evidence implicating the ankyrins, myD88, TUBE, PELLE or N5 in cell-death induction.

Death domains mediate protein-protein interactions with analogous sequences: the Fas/Apo1 and the p55-R death domains self associate and also bind to each other, while the death domain of MORT1/FADD interacts with the death domain of Fas/Apo1 (Refs 4-6). RIP and TRADD death domains bind to the corresponding regions in Fas and p55-R, respectively^{7,8}. The modes of action of the DAP kinase, p105, p100, ankyrin, myD88, TUBE, PELLE and N5 death domains are still unknown. However, it is tempting to speculate that they may also prompt association with death-domain-containing proteins. Since induction of NF- κ B activity by overexpression of p55-R and TRADD death domains is fully dependent on their structural integrity and ability to participate in protein-protein interactions⁸, it is possible that the direct binding of the death domain of TRADD (or of another downstream protein) and NF- κ B p100 and p105 or I κ B (γ or δ) death domains might be involved in the induction by TNF- α of NF- κ B activity. This speculation is in line with the fact that PELLE and TUBE, which are necessary for the nuclear transport of the DORSAL protein (the *Drosophila* analogue of NF- κ B), also possess death domains (Figs 1, 2). Moreover, TUBE and PELLE can bind to each other²⁴ (perhaps through their death domains). Together, these data raise the possibility of involvement of death domains in the signalling of NF- κ B activation.

It is not clear at present whether the transduction of the cytotoxic signal is the only function of death domains. We should consider that this motif might play a more general role than its name implies. Thus, it cannot be excluded that, in the context of other proteins, death domains could participate in transduction of signals unrelated to programmed cell death. It seems that, evolutionarily, the death domain represents a separate protein-protein interaction module that can be inserted into various signal transduction proteins such as receptors (Fas, p55-R, NGFR), kinases (DAP kinase, PELLE, RIP; however, RIP may not be a true kinase, since it lacks an ATP-binding site), potential adaptor proteins (MORT1/FADD, TRADD and TUBE), transcription factors (p100 and p105 NF- κ B), proteins of unknown function that are likely to be involved in signal transduction (myD88 or N5) and structural proteins, such as members of the ankyrin family.

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