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The death domain motif found in Fas (Apo-1) and TNF receptor is present in proteins involved in apoptosis and axonal guidance

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Abstract The interaction of Fas (Apo-1) and TNF receptor-1 with their respective ligands can lead to cell death. The so-called death domain, a sequence motif present in the cytoplasmic portion of the two receptors, has been identified as a critical structural element involved in signal transduction that leads to apoptosis. Here we describe several additional proteins which contain a death domain. Novel members of this family include proteins known to be implicated not only in apoptosis but also in neuron guidance.

Key words: Fas; Apo-1; TNF receptor; Death domain; Apoptosis: Neuron guidance; Reaper; Retinoblastoma; Unc; Ankyrin

1. Introduction

Apoptosis can be triggered by a myriad of external and internal stimuli. Amongst others, Fas/Apo-1/CD95 (Fas-R), TNF receptor 1 (TNF-R1), ICE and its family members, c-*myc*, p53, E2F-1 have been identified as positive mediators of apoptosis [1]. How all these distinct molecules trigger the same cell death program remains unknown, but an important role has been recently attributed to a sequence motif, the so-called death domain (DD), initially identified in the cytoplasmic segment of the homologous Fas-R and TNF-R1 [2]. Recently, several proteins interacting with the DD of these two receptors have been described which themselves contain DD-related sequences. Among these proteins, FADD/Mort 1 [3,4] and TRADD [5] uniquely associate with Fas-R and TNF-R1, respectively, while RIP [6] associates with Fas-R and to a lesser extend with TNF-R1. In all cases, binding to the respective receptor is mediated by a direct DD–DD interaction. Overexpression of these TNF-R1/Fas-R-associated proteins results in cell death. For this, we reasoned that the DD motif may be more generally implicated in cell death and searched protein databases for additional members of the DD protein family.

2. Materials and methods

Protein databases searched were SwissProt (Release 32) and genpept (Release 89+ updates, June 95) [7]. Database searches with query sequences were performed using the BLAST program [8]. Profiles were constructed from aligned sequences as described in [9], using the BLOSUM45 amino acid similarity matrix [10], a gap penalty of 2.1 and a gap extension penalty of 0.2. The profiles were used for protein database searches, using the methods described in [11]. Statistical significance of sequence-to-profile matches was assessed by regional shuffling [12] of the sequences, using a window size of 20 residues. Only scores of at least seven standard deviations above the average shuffled score were considered significant. In the course of the analysis, the profiles were iteratively refined by including significant matches into the profile-building process. For secondary structure prediction, the PHD mail server at the EMBL [13] was used. The profiles and programs used are available on request, they can also be accessed electronically by WWW, using the resource locator 'http://ulrec3.unil.ch/'.

3. Results and discussion

By searching protein data bases using generalized sequence profiles [11] based on the DD sequence of Fas-R (Fas/Apo-1/CD95) and TNF-R1, we have retrieved several additional proteins containing a DD motif (Fig. 1). Sequence comparison of all 13 distinct DDs discloses the hallmarks of this motif (Fig. 2). The DD, averaging 70–80 amino acids, is always localised near one end of the protein. Sequence homology is found at both ends, while the intervening middle region frequently contains insertions or deletions. There are several amino acid positions within the domain that are highly conserved, in particular Trp^{16}, Leu^{55}, Trp^{58}, Leu^{72} and Leu^{76}. An overall α-helical structure of the DD is predicted [13].

In addition to the sequence similarity, there exists evidence, both direct or indirect, implicating some of the new members of the DD family in cell death. The low affinity NGF receptor ($p75^{NGF-R}$) is a member of TNF receptor family and has been shown to constitutively induce cell death in serum-deprived neuronal cells [14]. DAP (death-associated protein)-kinase expression is indispensable for interferon-γ-mediated apoptosis in HeLa cells [15], since antisense inactivation of the respective mRNA protects cells from cell death. There may be a role for MyD88 in cell death as well, since its transcription has been shown to specifically occur upon terminal differentiation and growth arrest of myeloid precursors [16]. The resulting granulocyte lineage is short-lived as neutrophils die by apoptosis within 2–3 days.

P84 may also be a major player in cellular death. This nuclear protein was recently characterized by virtue of its interaction with the N-terminal region of the hypophosphorylated retinoblastoma gene product pRB [17]. pRB functions as a growth suppresser by binding the transcription factor E2F [18]. Viral oncoproteins such as E1A and E7 bind to identical sites, resulting in the liberation of the cell cycle-regulating proteins. One might predict, therefore, that in addition to its role as a repressor of proliferation, pRB could also act as regulator of apoptosis, by neutralizing the DD of p84. Indeed, pRB was recently shown to inhibit ionizing radiation-induced apoptosis [19], and mice deficient in pRB show increased levels of apoptosis in neuronal cells, leading to embryonic lethality [20].

Unc-5 [21] and *Unc-44* [22], an ankyrin-related gene product,

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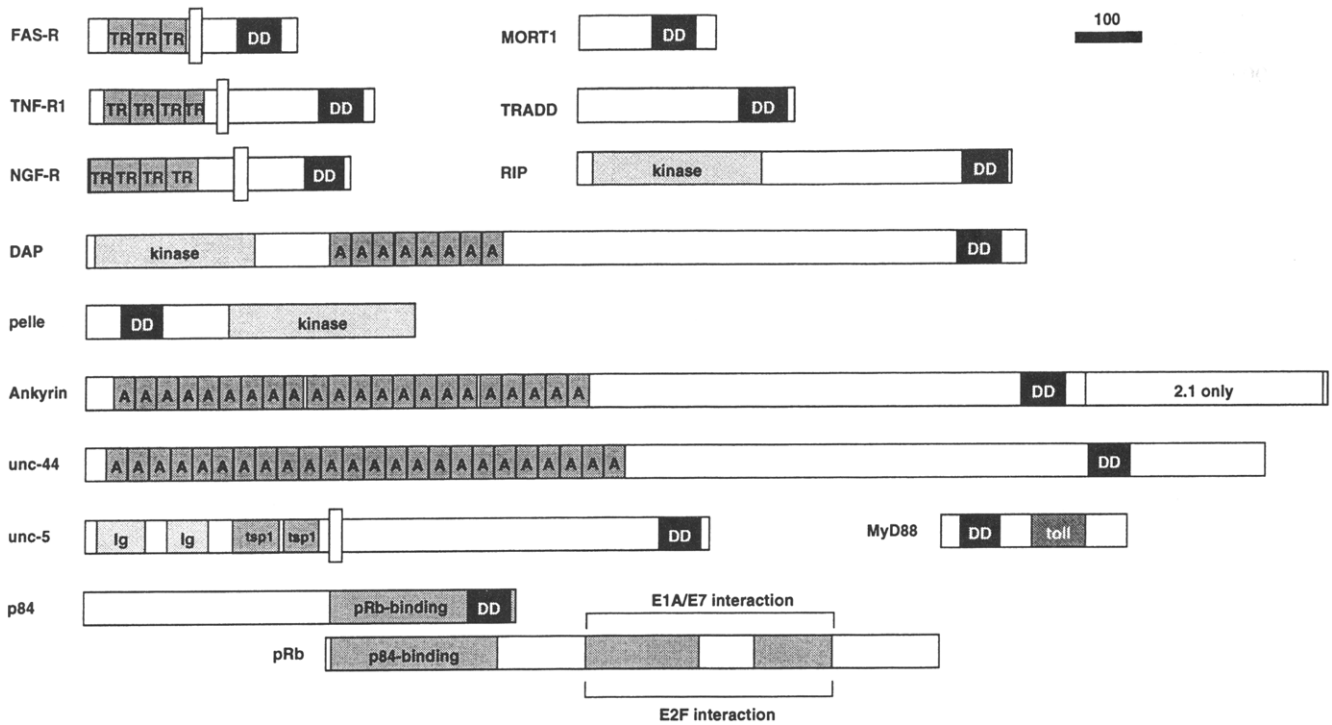

Fig. 1. The DD family members. Members are schematically drawn and include Fas-R, TNF-R1, NGF-R, Mort1/FADD, RIP, TRADD, DAP-kinase, ankyrin (2.1 and 2.2 isoforms), *pelle*, the ankyrin-like *Unc-44* and *Unc-5*, MyD88, retinoblastoma gene product (pRb)-binding protein p84. TR indicates the cysteine-rich domains found in TNF receptor family members, A refers to ankyrin repeats, *toll* to the domain present in *Drosophila toll* gene and IL-1 receptor, Ig to the immunoglobulin domain, and tsp1 to the thrombospondin type 1 domain. The vertical bars denote transmembrane segments. p85 and E1A/E7/E2F interaction sites of pRb (shaded boxes) are indicated.

are both required for guiding pioneering axons and migrating cells along the body wall in *Caenorhabditis elegans*, suggesting that their DD may be involved in microfilament reorganisation. It is noteworthy that cytoskeletal rearrangement is an early event of apoptosis. In contrast, the link between *Drosophila pelle* [23], a protein kinase required to establish dorsoventral polarity in the Drosophila embryo, and cell death remains unclear.

Our search has identified the presence of a DD in several proteins implicated in apoptosis, suggesting a general role for this domain in protein–protein interaction and cell death. The profile used, however, did not detect *reaper*, a *Drosophila* protein inducing cell death [1]. *Reaper* has been suggested to contain a DD based on a limited sequence similarity with the middle region of the DD of TNF-R1 [24]. This region, however, shows the weakest homology amongst DD members. More-

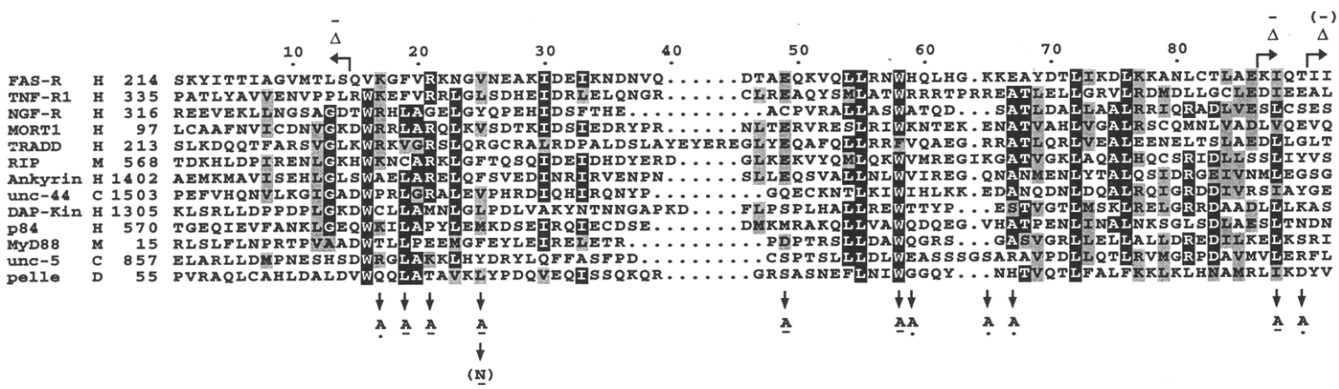

Fig. 2. Alignment of the DDs of Fas-R (Fas/Apo-1/CD95) (SP: FASA_HUMAN), TNF-R1 (SP: TNR1_HUMAN), $p75^{NGF-R}$ (SP: NGFR_HUMAN), human Mort-1/FADD (EM: X84709), human TRADD (EM: L41690), human RIP (EM:U25994), ankyrin (SP: ANK1_HUMAN), Unc-44 (EM: U21731), human DAP-kinase (EM: X76104), human p84 (EM: L36529), MyD88 (SP: MY88_MOUSE), C. elegans Unc-5 (EM: S47168), and Drosophila pelle (SP: KPEL_DROM). The length of the DB motif shown corresponds to the experimentally determined minimum length of the active TNF-R1 DB [25]. For MyD88, the exact translation start site is unknown. Loss of function (−) or non-relevant (·) amino acid replacements, introduced by alanine scanning mutagenesis in the DB of TNF-R1, are indicated [25]. The amino acid change which occurs in the Fas-R of *lpr*^{cg} mice resulting in an inactive receptor [2] is shown in brackets. Δ indicates non-functional deletion mutants constructed in the TNF-R or Fas-R (in brackets) [25–27]. Sequence segments were included in the alignment and used for profile refinement only if they matched the accepted profile with a residual error probability of $P < 0.05$

over, the *reaper* protein is shorter than the full length DD and does not include the conserved amino acids between residues 80 and 90 (see Fig. 2 for numbering) which were shown to be essential for the DD activity of the TNF-R1 [25].

Although the functional importance of the DD in these newly described proteins has yet to be proven, it is likely that their characterization will further our understanding of the molecular mechanisms underlying apoptosis.

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References

[1] Steller, H. (1995) Science 267, 1445–1449.
[2] Nagata, S. and Golstein, P. (1995) Science 267, 1449–1456
[3] Boldin, M.P., Varfolomeev, E.E., Pancer, C., Mett, I.L., Camonis, J H. and Wallach, D (1995) J. Biol. Chem. 270, 7795–7798.
[4] Chinnalyan, A.M., O'Rouke, K., Tewari, M. and Dixit, V.M. (1995) Cell 81, 505–512.
[5] Hsu, H., Xiong, J. and Goeddel, D V (1995) Cell 81, 495–504
[6] Stanger, B.Z., Leder, P., Lee, T.H., Kim, E. and Seed, B. (1995) Cell, 513–523
[7] Benson, D., Lipman, D.J. and Ostell, J. (1994) Nucleic Acids Res. 22, 3441–3444
[8] Altschul, S F, Gish, W., Miller, W, Myers, E.W. and Lipman, D.J. (1990) J. Mol Biol. 215, 403–410.
[9] Luthy, R., Xenarios, I. and Bucher, P (1994) Prot. Sci. 3, 139–146
[10] Henikoff, S. and Henikoff, J.G. (1992) Proc Natl. Acad. Sci. USA 89, 10915–10919.
[11] Bucher, P. and Bairoch, A. (1994) in: Proceedings Second International Conference on Intelligent Systems for Molecular Biology, pp. 53 (R., A., Brutlag, D., Karp, P., Lathrop, R. and Searls, D. eds.) AAAI Press, Menlo Park
[12] Pearson, W.R. and Lipman, D.J. (1988) Proc. Natl. Acad. Sci. USA 85, 2444–2448.
[13] Rost, B., Sander, C. and Schneider, R (1994) Comp. Appl. Biosci. 10, 53–60
[14] Rabizadeh, S., Oh, J., Zhong, L.T., Yang, J., Bitler, C.M., Butcher, L.L. and Bredesen, D.E. (1993) Science 261, 345–8.
[15] Deiss, L.P., Feinstein, E., Berissi, H., Cohen, O. and Kimchi, A. (1995) Genes Dev. 9, 15–30.
[16] Lord, K A., Hoffman-Liebermann, B. and Liebermann, D.A. (1990) Oncogene 5, 1095–7.
[17] Durfee, T., Mancini, M.A., Jones, D., Elledge, S.J and Lee, W.H. (1994) J Cell Biol. 127, 609–22.
[18] Weinberg, R.A. (1995) Cell 81, 323–330.
[19] Haaskogan, D.A., Kogan, S.C., Levi, D., Dazin, P., Tang, A., Fung, Y K.T. and Israel, M.A. (1995) EMBO J. 14, 461–472.
[20] Clarke, A.R., Maandag, E.R., van Roon, M., van der Lugt, N.M., van der Valk, M., Hooper, M.L., Berns, A. and te Riele, H. (1992) Nature 359, 328–30.
[21] Leung-Hagesteijn, C., Spence, A.M., Stern, B.D., Zhou, Y., Su, M.W., Hedgecock, E.M. and Culotti, J.G (1992) Cell 71, 289–99.
[22] Otsuka, A.J. et al. (1995) J. Cell Biol. 129, 1081–1092.
[23] Shelton, C.A. and Wasserman, S.A. (1993) Cell 72, 515–25.
[24] Golstein, P., Marguet, D and Depraetere, V (1995) Cell 81, 185–186.
[25] Tartaglia, L.A., Ayres, T.M., Wong, G.H. and Goeddel, D V (1993) Cell 74, 845–53.
[26] Boldin, M.P., Mett, I.L., Varfolomeev, E E., Chumakov, I., Shemeravni, Y., Camonis, J.H. and Wallach, D. (1995) J Biol Chem. 270, 387–391.
[27] Itoh, N and Nagata, S. (1993) J Biol Chem. 268, 10932-7